(54) Title: MICRO SYSTEM AND METHOD FOR FIELD MANIPULATION OF PARTICULES

(57) Abstract

The present invention pertains to a micro system comprising a system of operably linked, interconnected compartments wherein at least one reagent immobilised on at least one particle is capable of contacting an analyte comprised in a liquid carrier. The micro system comprises i) at least one particle with surface properties suitable for immobilising at least one reagent thereon, ii) at least one reagent suitable for being immobilised on the surface of the at least one particle, iii) a first compartment for storage of the at least one particle, iv) a second compartment in which the liquid sample may interact with the reagent immobilised on at least one particle, each of said first and second compartments having at least one opening for passing liquids between the compartment and the exterior, and v) means for subjecting at least part of the system to a field so as to move at least one particle between said first and said second compartment, and vi) a passage defined between said first compartment and said second compartment so as to allow at least one particle to be moved from one of said compartments to the other through said passage. There is also provided a method related.

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TITLE: MICRO SYSTEM AND METHOD FOR FIELD MANIPULATION OF
PARTICLES.

5   TECHNICAL FIELD OF THE INVENTION

The present invention concerns manipulation of particles by using fields to which
the particles are susceptible, primarily for analytical purposes or for performing
synthesis. In particular, particles that are susceptible to magnetic fields are

10   considered.

BACKGROUND OF THE INVENTION

15   The automation of reagent handling is a central issue in the development of
systems for chemical analysis and synthesis. Higher degree of automation expands
the possibility of chemical analysis and increases the availability of chemical
information. The benefit of this comes to medical diagnostics, environmental
monitoring, process control and other disciplines, that require detailed chemical

20   information. Typically, reagent handling is automated either by robotics, by pressure
driven flow systems or by systems based on electrophoretic forces.

Miniaturisation added to the automation gives advantages as better flow control
(pressure driven systems work exclusively in the laminar flow regime), increased

25   efficiency of chromatographic processes (e.g. capillary electrophoresis) due to
increased surface-to-volume ratio, reduced reagent costs, and the possibility to
make portable, sensor-like systems. The acronym "μ -TAS" (micro total analysis
system) is used for such systems and covers micro systems with automation of every
operational step in a chemical assay. A flow injection analysis (FIA) system is another

30   approach to miniaturised reagent handling and eventually analysis.

A number of systems for analysing and/or manipulating target particles have been
described in the prior art.

WO 98/10267 discloses a flow system wherein the flow of a fluid comprising particle species including target particles to be analysed may be diverted into different channels in order to achieve a separation of the target particles. The present
5   invention does not employ a dynamic flow of a liquid in order to sort or separate target particles from non-target particles. The system according to the present invention makes it possible to manipulate particles in such a way that there is substantially no convection between liquids present in different compartments of the system. Consequently, the system according to the present invention is not a flow
10   system such as the system disclosed in WO 98/10267.

WO 93/22053 discloses another flow system and is also not pertinent to the present invention. Furthermore, in the invention disclosed in WO 93/22053, a chemical reaction and a detection reaction takes place in the same compartment. In contrast,
15   the present invention employs different compartments for chemical reactions and detection reactions. This facilitates a superior and much improved separation of target particles and reaction mixture and/or sample. Another advantage of the present invention is the inclusion of further separate purification steps and detection steps following the separation of target particles and non-target particles present in
20   the sample.

US 4,868,130 discloses a macro system for separating target particles from non-target particles. It does not disclose a micro system. Accordingly, much larger sample volumes are needed and substantially more convection between liquids in
25   separate compartments takes place. The present invention discloses a microsystem for manipulating particles in such a way that there is substantially no convection of liquids present in different compartments of the system.

30   SUMMARY OF THE INVENTION

The present invention discloses a micro system and a method for field manipulation of particles capable of being used as a detection means, e.g. in a diagnostic

method. In particular, the micro system according to the present invention is a micro system that facilitates manipulation of particles in such a way that there is substantially no convection between liquids present in different compartments of the micro system.

In particular, the micro system does neither employ nor depend on an essentially unidirectional flow of a fluid in order to generate a translocation of a particle from one compartment of a system to another. The micro system according to the invention can be described as a system comprising a plurality of compartments, wherein the compartments in between which a particle is moved can be perceived, at least part of the time said movement takes place, as a substantially closed, non-flow system. Accordingly, the present invention is concerned with the movement of particles in a substantially "non-flow" liquid system. In this respect it is particularly preferred to use a micro system, as such a system tends to stabilise liquid samples and reduce and/or eliminate any convection. This in turn leads to an improved and predeterminable movement of particles within said micro system comprising one or more essentially static and "non-flow" liquids.

The micro system according to the invention represents an improvement of state of the art systems for analysis and/or detection. The present micro system has an improved sensitivity and employs different compartments for performing i) a chemical reaction between a) a specific reagent that is immobilised on a particle susceptible of manipulation by a field, and b) a target substance such as e.g. a biological cell or a part thereof including antigenic and/or immunogenic determinants located e.g. extracellularly, and ii) a detection reaction for detecting said target substance and said specific reagent in reactive contact with one another.

In contrast to the well-recognised problem of convection associated with macro systems, where convection may occur within a single compartment, the problem of convection is usually minimised if not eliminated altogether for micro systems.

Ready-to-use, disposable micro systems or parts thereof will often be stored in the cold prior to use in order to preserve the quality of the chemicals used and to try to prolong the shelf life of the system. The temperature is usually raised to a working temperature just prior to use, and this change of temperature creates temperature
5 gradients within the system and within separate compartments of the system that will lead to convection in a macro system, but not in the micro systems according to the invention.

Means of detection such as e.g. laser excitation or electrical currents may also
10 significantly contribute to the development of temperature gradients and subsequent convection in a macro system. The effect is much less pronounced if not altogether negligible for a micro system under practical circumstances.

Furthermore, the absence of a tendency of convection within a single compartment
15 promotes micro system particle movement in a well-defined and predetermined manner. This promotes an accurate calculation of a contact time for each compartment, and this in turn significantly reduces the stochastic error on the result of an analysis.

20 Accordingly, the present invention facilitates a much-improved separation of target substances from non-target substances. It is possible according to the present invention to detect target substances in a sample such as a biological sample substantially without a number of in vitro steps normally applied in order to prepare a sample for analysis. This is achieved by placing a sample preferably in an
25 substantially non-manipulated condition in a compartment of the micro system according to the invention and contact said sample with any reagent of interest including highly specific reagents such as antibodies, ligands or receptor moieties that are immobilised on particles susceptible of manipulation by a field such as e.g. a magnetic field. When being manipulated by said field, the particles will deliver the
30 specific reagent or reagents to the sample, where each reagent is brought into reactive contact with a target substance in the form of a binding partner capable of reacting with said reagent.

By further field manipulation of said particles it is possible to separate the target substances from non-target substances contained in the analysed sample. The contacting of the target substances by the reagent for which they are specific and the separation of target substances from non-target substances preferably takes

5   place under conditions that mimic or are at least functionally similar to in vivo conditions characterising said sample prior to isolation. Following separation of target substances from non-target substances it is possible to further include purification steps and detection steps in order to increase the sensitivity of the method and optionally optimise the quantification of target substances. It is

10 particularly preferred to be able to specifically isolate a target substance and at the same time be able to quantify the amount of target substance present in the sample. It may be of vital importance prior to or during surgery to be able to quickly and accurately monitor e.g. cellular developments and/or antibody reactions. This can be achieved by using the micro system and the methods according to the

15 present invention.

It is a further advantage of the present invention that it may easily be automated without the need for advanced and costly liquid handling systems and robotics. Consequently, the methods of e.g. diagnosing a target substance in a biological

20 sample that are described herein are both quick and cheap and rapidly generate test results that are both accurate and quantifiable.

The invention in one presently preferred embodiment comprises a diagnostic method and a disposable, ready-to-use devise for use in said method. The

25 disposable nature of the micro system ensures a prompt and efficient elimination of any harmful substances that may have been contained in the analysed sample. It is even possible to discard the disposable micro system before the test result is available, and costs for cleaning and decontamination can be kept at a minimum.

30 The invention is not limited to the analysis of biological samples under conditions essentially similar to in vivo conditions, even though this form of analysis represents one presently preferred embodiment of the invention. The person skilled in the art will know how to obtain and treat samples including biological samples in a number

of ways prior to analysis by using the micro system and the methods according to the present invention.

Controlled manipulation and measurement of magnetic particles

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The key concept of the present invention is to do reagent handling by immobilising one or more reagents on one or more particles and move the particles by fields to which the particles are susceptible, such as e.g. magnetic fields, gravitational fields, an apparent field applied by centrifugation or electric fields by which the particles

10   are moved by electrophoresis or dielectrophoresis. To control the particles, the fields preferably has to be created with high degree of repeatability and with a complexity that allows for particle movement within and between the compartments as desired. It is particularly preferred that the fields are well defined, as this helps to control particle movement.

15

By moving the reagent-coated particle between and within compartments of different chemical environments in a controlled and well-timed manner, a multitude of analytical chemical operations can be performed. Said compartment may have different functions, such as sample interaction, complexing measurable probes to

20   the bound analyte (on a particle), detection of specific properties of the particles, making derivatives of the coated chemicals (synthesis) or washing the particles.

The particles have a diameter typically in the range from 0,5 micro meter to 1 mm, depending on the process. Accordingly, the particles may have a diameter of more

25   than for example 1 micro meter, such as more than 2 micro meter, for example more than 4 micro meter, such as more than 6 micro meter, for example more than 8 micro meter, such as more than 10 micro meter, for example more than 15 micro meter, such as more than 20 micro meter, for example more than 25 micro meter, such as more than 30 micro meter, such as more than 35 micro meter, for example

30   more than 40 micro meter, such as more than 50 micro meter, for example more than 60 micro meter, such as more than 70 micro meter, for example more than 80 micro meter, such as more than 90 micro meter, for example more than 100 micro meter, such as more than 125 micro meter, for example fore than 150 micro meter,

such as more than 200 micro meter, for example more than 250 micro meter, such as more than 300 micro meter, for example more than 400 micro meter, such as more than 500 micro meter, for example more than 600 micro meter, such as more than 700 micro meter, for example more than 800 micro meter, such as more than 5 900 micro meter, and less than 1000 micro meter.

It is particularly preferred that the particles have a diameter of typically less than 1000 micro meter, such as a diameter of more than 0.5 micro meter and less than for example 1 micro meter, such as less than 2 micro meter, for example less than 10 4 micro meter, such as less than 6 micro meter, for example less than 8 micro meter, such as less than 10 micro meter, for example less than 15 micro meter, such as less than 20 micro meter, for example less than 25 micro meter, such as less than 30 micro meter, such as less than 35 micro meter, for example less than 40 micro meter, such as less than 50 micro meter, for example less than 60 micro 15 meter, such as less than 70 micro meter, for example less than 80 micro meter, such as less than 90 micro meter, for example less than 100 micro meter, such as less than 125 micro meter, for example fore than 150 micro meter, such as less than 200 micro meter, for example less than 250 micro meter, such as less than 300 micro meter, for example less than 400 micro meter, such as less than 500 20 micro meter, for example less than 600 micro meter, such as less than 700 micro meter, for example less than 800 micro meter, such as less than 900 micro meter.

The particles may preferably be made of a magnetically responsive material (MRM) and optionally one or several coating materials, to make the particles both 25 magnetically responsive and suitable for reagent coating. Many different kinds of magnetic particles are commercially available from companies such as Dynal, Bangs Laboratories and Miltenyi.

Non exclusive examples of the MRM's in the particles could be alloys, oxides, 30 sulfides and borides of iron, nickel, cobalt and some rare earth elements. The MRM can either be ferromagnetic, paramagnetic or superparamagnetic. For some embodiments paramagnetic and even more superparamagnetic particles are

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preferably used since they loose their own magnetism as soon as the external magnetic field is removed.

Many different bulk materials for surrounding the MRM have been reported. A few
5 examples are polystyrene, starch, dextran and poly-methyl-methacrylate. The bulk material itself, additives or surface modifications can be used for coating the particles with reagents.

The magnetic particles can be coated with many types of reagent, for example an
10 immunogenic determinant, an antigenic determinant, an epitope; an antibody, that will bind a certain antigen or a binding fragment thereof (molecular or particular); double or single stranded DNA or RNA or LNA or PNA, preferably DNA capable of being used as a template for amplification by e.g. PCR (Polymerase Chain Reaction) or in hybridisation assays; drug receptors; biotin or streptavidin; chelates;
15 pH or red-ox sensitive indicators; precursors of a desired product for synthesis. The reagent can be bound to the particle in every way known in the art of immobilisation. For example covalent bonds, ionic interactions, and non-polar interactions or combinations thereof have been employed.

20 Other embodiments of the invention use chemically sensitive, magnetically responsive eukaryote or prokaryote organisms such as magneto-bacteria for magnetic particles. The microorganisms can either have a native magnetic responsiveness (magneto-bacteria or red blood cells with deoxy-haemoglobin) or have acquired the feature artificially.
25

When the invention is used for analysis, the analytes are strongly related to the reagent coating of the magnetic particles. Some analytes that relates to reagent mentioned before are be enzymes, other proteins, hormones, vitamins, cells, vira, drugs (are bound by antibodies or drug receptors); DNA and RNA (are bound by
30 DNA- and RNA-probes); metal ions (are bound by chelating agents); pH and red-ox potential (from pH or red-ox indicators).

Compartments

A fluid such as a liquid in the form of a liquid carrier that is comprised within a
5   compartment is substantially physically unaffected by the contents and action in the
adjacent compartment within a time scale relevant for the processes.

A compartment is confined from the surroundings by compartment walls except for
an optional number of inlets or outlets and an optional number of interfaces that
10   interconnect the compartment to other compartments. The compartment walls may
be liquid, solid or gas phase. The inlets/outlets are meant to replenish the
compartment with whatever chemical solution it needs to perform its function in the
analysis/synthesis systems.

15   According to the invention, a typical analysis or synthesis system consists of many
different compartments. Some are mentioned here as examples.

Particle storage/supply compartments

20   Before performing the assay or synthesis the particles are situated in compartment
for particles storage. Besides the particles, the compartment may contain a pH-
buffer and optional additives to assure long time stability of the reagent and the
immobilisation. The pH is typically in the range of from 2 to 13, depending on the
reagent, and in most cases preferably in the range of from 5 to 9, such as from 5.5
25   to 8.5, for example from 6 to 8, such as from 6.5 to 7.5, such as around neutral pH.

Sample interaction compartments

During analysis the particles have to be allowed to interact with the sample and this
30   interaction is accomplished in the 'sample interaction compartment'. The
compartment typically has one inlet and one outlet for introducing the sample and
two interfaces: one for the particle supply and one for a compartment of further
analysis. During the sample interaction the reagents on the magnetic particles will

either be chanced due to the chemical environment of the sample or bind certain analytes of the sample.

The volume of the compartment for sample interaction should have a size that

5 allows for detection of the analyte. The volume range should be within 1 pL to 10 mL. In the case of analysis the term 'sample' is often used for an amount of liquid (or solid or gas) that is representative of the unknown liquid (or solid or gas) in the sense that the analytes from the unknown liquid should be present in sample in reproducible amounts.

10

One of the following reactions may happen in the compartment for sample interaction: the magnetic particles may bind analyte from the sample, the analyte may chance the surface reagents of the magnetic particles (without separating the analyte from the sample), a reaction between the immobilised reagents and the

15 analytes takes place via an intermediate species.

Secondary interaction compartments

In the case of analysis the purpose of the secondary interaction compartment is to

20 perform a reaction that makes the changes of the particles during sample interaction detectable. This reaction could as examples and not limitations be binding detectable probes (fluorescent antibody, fluorescent DNA, fluorescent chelates, etc.) to either unaltered particle bound reagent, analyte that is bound to particle bound reagent or particle bound reagent that has been changed due to the

25 sample interaction.

In the case of synthesis the secondary interaction is to alter the reagents on the surface of the magnetic particle with the purpose of building up new molecules. These alterations could for example be the controlled build-up of polymers like

30 proteins or DNA/RNA or 'point of care' synthesis of drugs or reagents.

Compartment interfaces

The interface can be one of the following: a mixing zone of the aqueous liquids of two or more compartments; a gas plug between two or more compartments; a plug

5   of an insoluble, organic liquid between two or more compartments; a plug of solid matter that may be melted/turned into liquid at will; an actuatable or passive membrane between two or more compartments; a breakable seal between two or more compartments.

10   The compartments could also be assembled or aligned just before the analysis has to be performed. One interesting embodiment of this would have a least one position for filling the compartments, one position for storing the system and one position for performing the analysis. Optionally a position for filling the sample in the system could be included.

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DETAILED DESCRIPTION OF THE INVENTION

One particularly preferred aspect of the invention concerns a micro system

20   comprising a system of operably linked, interconnected compartments wherein at least one reagent immobilised on at least one particle is capable of contacting an analyte comprised in a liquid carrier, said micro system comprising

i) at least one particle with surface properties suitable for immobilising at least one

25   reagent thereon,

ii) at least one reagent suitable for being immobilised on the surface of at least one particle,

30   iii) a first compartment preferably for storage of at least one particle,

iv) a second compartment in which the liquid sample may interact with the reagent immobilised on at least one particle, each of said first and second compartments

having at least one opening for passing liquids between the compartment and the exterior,

v) means for subjecting at least part of the system to a field so as to move at least
5   one particle between said first and said second compartment, and

vi) a passage defined between said first compartment and said second compartment so as to allow at least one particle to be moved from one of said compartments to the other through said passage.

10

The terms micro system and device are used interchangeably herein and are used in the context of down-scaled systems or systems capable of operating in a microscale or mesoscale environment.

15   It is particularly preferred that said system facilitates under practical circumstances a movement of at least one particle generated by said force in such a way that substantially no convection of a liquid carrier in said first compartment and a further liquid carrier in a second compartment takes place during said particle movement and/or analyte contacting by said reagent.

20

Three types of convection may theoretically occur in connection with the use of the micro system and/or the methods according to the present invention. For the sake of clarification, these types of convection have been illustrated in Figure 20. Figure 20 illustrates two compartments comprising two liquids (71 and 72) operably linked
25   by means of an interconnection in the form of a passage 73. The type of convection perceived as flow according to the present invention is illustrated (74) and involves a substantially uni-directional, bulk movement of liquid through both compartments. According to the present invention, substantially no flow is present at least part of the time during which particle movement takes place.

30

If e.g. liquids 71 and 72 are separated and have different densities, a phenomenon termed inter-compartment convection (75) will take place. This is illustrated in Fig-

ure 20 as an ellipsoidal bulk movement that crosses the passage between the liquids.

Convection within the same compartment is called intra-compartment convection

5   (76) and may take place e.g. when a heat gradient is generated within a compartment. Downscaling compartment dimensions generally reduces and/or eliminates intra- and inter-convection as defined herein above.

Densities of liquids should preferably differ by not more than about 30%, preferably

10   by less than about 20%, more preferred by less than about 10%, and does not differ substantially in one most preferred embodiment of the invention.

The compartments are in one preferred embodiment tubes, which are relatively long compared to their diameter or cross-section. The cross-section should pref-

15   erably be less than about 1 cm, more preferably from about 1000 µm to about 10 µm, more preferred from about 800 µm to about 100 µm, and most preferred about 600 µm. The term "microscale" refers to such small cross-sections. The cross-section should be kept small in order to minimize convection. Note that, due to the microscale of the compartments relevant for the present invention, flow can be pres-

20   ent without turbulence.

The length of the compartments may be any convenient length, preferably about between 1 m and 0,05 cm, more preferably about between 10 cm and 1 mm, more preferred about between 5 cm and 1 cm, and most preferred about 2 cm.

25

The velocity of the one or more particles when being moved, e.g. in a compartment or from one compartment to another, should preferably be between from about 1 µm/s to about 1 cm/s, more preferably from about 2 µm/s to about 1 mm/s, more preferred from about 3 µm/s to about 100 µm/s, preferably from about 5 µm/s to

30   about 50 µm/s, and is in one most preferred embodiment of the present invention about 30 µm/s.

The velocity of the liquid is preferably less than or about 1/10 of the particle velocity, more preferred less than or about 1/100 of the particle velocity and in one best mode the velocity of the liquid is zero.

5 The time scale of an experiment according to the present invention varies from seconds to hours. It is preferably less than about 30 minutes, preferably from about 2 to about 10 minutes and in one best mode about 3 minutes. The person skilled in the art will be able to adjust the time scale without undue experimentation. Longer time means longer reaction time, while shorter time gives faster experiment. The

10 particle concentration should be balanced with the time used for aligning the particles. This balance depends on the actual channel structure, the magnet setup, the magnetic particles and the pump sequences that are used to fill the compartments.

The liquids according to the invention may be further characterised by a Rayleigh

15 number that describes the tendency of a liquid to contribute to either intra- or inter-compartment convection due to e.g. a physical difference between said liquids attributable to e.g. temperature and density and the like. Below a Rayleigh number of around 1700, the separated liquids will be metastable even if e.g. a colder (heavier) liquid is placed on top. Since the Rayleigh number is proportional to the third power

20 of the distance between e.g. the warmer and colder part of the liquid. Consequently, downscaling leading to smaller distances will have a highly stabilising effect on the system.

In another preferred aspect of the invention there is provided a method of moving a

25 particle comprising at least one reagent immobilised thereon into a liquid sample that is contained in a micro system comprising a system comprising a plurality of operatively linked compartments, the method comprising the steps of

i) providing at least one particle with at least one reagent immobilised thereon, and

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ii) entering said particle into a first compartment, and

iii) entering a liquid carrier into said first compartment, and

iv) entering a further liquid carrier into a second compartment, and

v) subjecting said micro system to a field exerting a force on at least one particle

5   susceptible to said field, and

vi) moving by means of said force at least one particle from said liquid carrier
comprised in said first compartment into said further liquid carrier comprised in said
second compartment, wherein said movement of at least one particle generated by

10   said force is such that substantially no convection of said liquid carrier and said
further liquid carrier takes place during particle movement and/or analyte contacting
by said reagent, and optionally

vii) contacting an analyte comprised in said further liquid carrier with said reagent

15   on at least one particle.

It is particularly preferred that said first and second compartments of said micro
system form a substantially closed, non-flow system at least during part of the time
said movement of at least one particle takes place.

20

In one embodiment of this aspect of the invention, said further liquid carrier is a
sample for analysis putatively comprising an analyte capable of being contacted by
said reagent. Accordingly, the method in one embodiment also comprises the step
of contacting an analyte comprised in said further liquid carrier with said reagent on

25   at least one particle.

In one particularly preferred embodiment according to this aspect of the invention,
the method comprises at least one further step of washing and/or purifying and/or
detecting said analyte in said first compartment comprising said liquid carrier and

30   optionally an amount of said further liquid carrier that does not interfere with the
efficacy of said step of washing and/or purifying and/or detecting said analyte, said
method comprising the even further step of moving said reagent contacting said
analyte comprised in said sample for analysis comprised in said further liquid

carrier, from said further liquid carrier comprised in said second compartment into said first compartment comprising said liquid carrier.

In another preferred aspect of the invention there is provided a method of moving a

5   particle comprising at least one reagent immobilised thereon into a liquid sample that is contained in a micro system comprising a system comprising a plurality of operatively linked compartments, the method comprising the steps of

i) providing at least one particle with at least one reagent immobilised thereon, and

10

ii) entering said particle into a first compartment, and

iii) entering a liquid carrier into said first compartment, and optionally

15   iv) contacting an analyte comprised in said liquid carrier with said reagent on at least one particle, and

v) entering a further liquid carrier into a second compartment, and

20   vi) subjecting said micro system to a field exerting a force on at least one particle susceptible to said field, and

vii) moving by means of said force at least one particle comprising a reagent contacting said liquid carrier comprised in said first compartment into said further

25   liquid carrier comprised in said second compartment, wherein said movement of at least one particle generated by said force is such that substantially no convection of said liquid carrier and said further liquid carrier takes place during particle movement and/or analyte contacting by said reagent.

30   The embodiments of the invention pertaining to methods of moving a particle are preferably performed by using a micro system as described herein and in the claims.

In one particularly preferred embodiment of this method, the liquid carrier is a sample for analysis putatively comprising an analyte capable of being contacted by said reagent. Accordingly, this method comprises a step of contacting an analyte comprised in said liquid carrier with said reagent on at least one particle

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In one preferred embodiment according to this method, the further liquid carrier is entered into said second compartment prior to or simultaneously with said analyte in said liquid carrier being contacted with said reagent on at least one particle. In another preferred embodiment, said particle and said liquid carrier are entered into

10   said first compartment either at least essentially simultaneously or sequentially in any order.

It is preferred that the particle is disposable and more preferably, the particle is reconstitutable from a long term storage stable condition such as a frozen,

15   cryoprotected, or freeze dried condition, prior to being entered into said first compartment.

The liquid carrier and/or further liquid carrier is preferably selected from the group consisting of water, saline, any physiologically acceptable aqueous solvent, any

20   pharmaceutically acceptable aqueous solvent, any organic solvent, including any mixture thereof.

The reagent is preferably selected from the group consisting of a nucleic acid such as a DNA, RNA, LNA or PNA molecule, including any derivative or part thereof, a

25   polypeptide, including any derivative or part thereof including peptides and epi-topes, a receptor moiety such as a receptor capable of binding a cell differentiation factor such as a cytokine or a lymphokine, an antibody including a chimeric anti-body, a heterodimeric antibody, and a monoclonal antibody, including any binding fragments thereof. Reagent systems that result in a detectable signal upon binding

30   of analyte to the reagent, such as the Taq system from Perkin Elmer, are one type of preferred reagent systems.

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It is particularly preferred that one embodiment of the method according to the invention comprises at least one further step of washing and/or purifying and/or detecting said analyte contacted by said reagent, with the proviso that said at least one further step does not take place in said first compartment.

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It is also preferred that the movement of at least one particle generated by the force to which said particle is susceptible is such that substantially no convection of said liquid carrier and/or said further liquid carrier takes place during particle movement and/or analyte contacting by said reagent.

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The analyte present in said liquid carrier or said further liquid carrier and contactable by said reagent is preferably a biological organism, or a part thereof, selected from the group consisting of a cell, an infectious agent including a virus, and a parasite, including any part or combination thereof.

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It is particularly preferred that the organism is a mammalian organism such as a human or animal organism, such as a human or animal cell, including any derivative thereof, or a virus or parasite, including any parasitic fungi, capable of being harboured in or replicated in a human or animal cell, or a derivative thereof.

20   The cell, virus or parasite is preferably pathogenic or potentially lethal.

The organism may also be a microbial organism such as a eukaryotic or prokaryotic microbial organism, and again, a pathogenic microbial organism and/or a potentially lethal microbial organism is particularly preferred. The terms pathogenic and

25   potentially lethal are easily recognisable by the skilled person as traits giving raise to at least pain, irritation, and a harmful infection, if not giving raise to a full blown disease or a serious illness, following habitation of such a pathogenic and/or potentially lethal organism in e.g. a human or animal body. However, the analyte may also be an antigen or an antibody indicative of a predetermined cell type or cell

30   line or differentiated cell line such as e.g. a cancer cell line or the development of metastasis.

In one interesting embodiment of the invention there is provided a method according to the invention comprising the further step of performing in at least one of said compartments a method of amplifying a biological compound by a plurality of thermo cyclic reactions at predetermined temperatures, such as reactions

5   suitable for annealing nucleic acids, extension reactions suitable for synthesising a nucleic acid, and denaturing reactions suitable for separating synthesised double stranded nucleic acids.

One particularly interesting aspect of the invention pertains to a method of

10   diagnosing a condition in an individual by detecting an analyte in a sample,

said diagnostic method comprising providing a sample from said individual and a method of detecting in said sample an analyte, the presence of which is an indication of said individual having contracted said condition,

15

said method of detection further comprising the steps of

i) moving a particle comprising at least one reagent immobilised thereon into a liquid sample that is contained in a micro system comprising a system comprising a

20   plurality of operatively linked compartments according to the method according to any method described herein and in the claims,

ii) contacting said reagent with said analyte comprised in a sample in the form of said liquid carrier or said further liquid carrier for the purpose of

25

iii) detecting diagnostically said analyte contacted by said reagent, and

iv) Diagnosing said condition.

30   In yet another preferred aspect of the invention there is provided a method of moving a particle with at least one reagent immobilised thereon into a liquid sample that is contained in a system comprising a first compartment that is interconnected

with a second compartment, each of which having an opening defined therein, the method comprising the steps of

entering at least one particle with at least one reagent immobilised thereon into the

5   first compartment,

entering the liquid sample into the second compartment,

subjecting the system to a field to which the at least one particle is susceptible

10   whereby the at least one particle is moved into the second compartment by a force exerted on the at least one particle by the field so that the reagent on the at least one particle may interact with the content of the liquid sample contained in the second compartment in such a way that substantially no convection of the liquids in the system takes place during said movement and said interaction.

15

The invention also concerns a device comprising a system in which the above described method may be carried out.

A device according to the invention comprises a system of compartments in which

20   the content of a liquid sample may interact with at least one reagent immobilised on at least one particle, the device comprises

at least one particle with surface properties suitable for immobilising at least one reagent thereon,

25

at least one reagent suitable for being immobilised on the surface of the at least one particle,

a first compartment for storage of the at least one particle,

30

a second compartment in which the liquid sample may interact with the reagent immobilised on the at least one particle, each of said first and second compartment

having at least one opening for passing liquids between the compartment and the exterior,

means for subjecting at least a part of the system to a field so as to move the at least one particle between the first and the second compartment, and

a passage defined between the first compartment and the second compartment so as to allow the at least one particle to be moved from one compartment into the other compartment through the passage.

The second compartment of the system may further comprise a second opening for passing liquids between the compartment and the exterior so that the liquid contained in the second compartment may be replaced with another liquid without affecting the contents of the other compartments of the system.

In a preferred embodiment, the step of subjecting the system to a field comprises the step of positioning field generating means for generation of a field that is subjected to at least a part of the system at the system.

The step of subjecting the system to a field may comprise the step of generating a magnetic field, e.g. by positioning an electromagnet at the system and subjecting the system to a field by activating the electromagnet with an electric current.

The magnetic system may be created with one or more permanent magnets or electromagnets. The field generating means may both be integrated within the system or applied externally. The magnetic field may be constant in time (e.g. stationary permanent magnet or DC electromagnet) or may be changing in time (could be due electromagnets with changing currents, moving permanent magnets or moving DC electromagnets). Zones of material with ferro-, dia-, para- or superparamagnetic can be deposited within or outside the compartment complex to focus or distort the magnetic field.

Bigger compartment dimensions, smaller particle sizes and lower content of magnetic material in the particles reduce the particle velocity. Therefore the relatively small compartments and relatively large particles will allow for a simpler and less power consuming magnetic system.

5

The speed of a magnetic particle in a time constant magnetic field from a permanent or electromagnet will accelerate as it approached the magnet. A substantially constant particle speed can be achieved by moving the permanent magnet or decreasing the current through the electromagnet as the particles comes

10   closer.

In an alternative embodiment, the step of subjecting the system to a field comprises the step of positioning two electrodes in electrical contact with the liquid in the system and subjecting the system to a field by supplying an electric potential

15   between the two electrodes so that the at least one particle is moved by electrophoresis.

In an further alternative embodiment, the step of subjecting the system to a field comprises the step of positioning field generating means at the system comprises

20   positioning two electrodes at the system in such a way that they are not in electrical contact with the liquid in the system and subjecting the system to a field by supplying an electric potential between the two electrodes so that the at least one particle is moved by dielectrophoresis.

25   The system may also be subjected to an apparent field by performing centrifugation of the system so as to move the at least one particle, or the system may be subjected to the field of gravitation, e.g. by changing the vertical orientation of the system when a movement of the at least one particle is demanded. The at least one particle may in the case of a gravitational field be moved either towards or against

30   the gravitational field, depending on the ration between buoyancy forces and gravitational forces on the at least one particle.

Any one, alone or in combination with one or more of the other of the above-mentioned fields may also be applied to the device comprising the system for enabling the steps of the method involving movement of the at least one particle. Accordingly, the at least one particle may be moved by a single or a combination of
5   the above-mentioned fields.

The method of moving the particle into a liquid sample preferably further comprises the step of monitoring properties of the at least one particle during the interaction and/or monitoring properties of the at least one particle after the interaction. The
10   device according to the invention may comprise detection means for detecting properties of the at least one reagent immobilised on the surface of the at least one particle.

These properties could e.g. be fluorescent properties that may be detected with a
15   photomultiplier or a CCD-array combined with a suitable objective or could be magnetic properties that may be detected with a Hall-sensor.

The detection of chemical properties in a compartment may employ all the methods known in the art of chemical detection. According to the invention the luminescence
20   methods (fluorescence, phosphorescence, chemi- or bioluminescence) are specially preferred. Regarding fluorescence and phosphorescence, the compartment needs to be transparent to allow for electromagnetic excitation of the relevant molecules and getting the emitted light to the detector (assuming external detection and excitation source - these might also be integrated). Furthermore,
25   materials of the compartment that are not autofluorescent within the wavelengths of the measured molecules are preferred when fluorescence (molecules with short-lived excited states) is detected.

The detection system may both measure the particles as they move through the
30   detection volume or make measurements on an area where the particles are made to settle. The latter approach allows for detection with confocal fluorescence microscopy giving a high signal/noise ratio.

24

An alternative to the optical methods is electrochemical detection principles as conductivity, amperometry and potentiometry. External or integrated electrodes for these measurements may be used in for the detection function in a compartment. Other types of detectors such as surface acoustic wave devices may also be used.

5

The device with the system may, according to the invention, comprise at least one field generating means adapted to apply a field to at least a part of the system, and the at least one particle being at least partly made from a material susceptible to the generated field. The generated field may be a magnetic field and the field
10 generating means may comprise at least one electromagnet and/or at least one permanent magnet.

Alternatively, the device may comprise field generating means comprising two
15 electrodes in electrical contact with the liquid in the system so that the field generating means are activated by applying an electrical potential difference between the two electrodes and the at least one particle is moved by electrophoresis.

20 In a further alternative embodiment of the invention, the device has field generating means that comprises two electrodes which are not in electrical contact with the liquid in the system so that the field generating means are activated by applying an electrical potential difference between the two electrodes and the at least one particle is moved by dielectrophoresis.
25

In a still further embodiment of the invention, the device comprises field generating means that are adapted for moving the at least one particle both back and forth between compartments between which a passage is defined.

30 Optionally, the system further comprises a third compartment for detection of properties of the at least one particle, that is interconnected with the second compartment, and the method further comprises the step of moving the at least one particle by means of the field into the third compartment so as to perform the

monitoring of the properties of the at least one particle when the at least one particle is situated in the third compartment.

The optional third compartment for performing the detection of the properties of the
5   at least one reagent immobilised on the surface of the at least one particle with the detection means may comprise

an opening for passing liquids between the compartment and the exterior,

10   an area that is transparent to allow of optical access from the exterior to the interior of the compartment, and

a passage defined between the second compartment and the third compartment so as to allow particles to be moved between the second compartment and the third
15   compartment,
the means for subjecting at least a part of the system to a field being adapted for moving the at least one particle between the second compartment and the third compartment by said field.

20   The system may further comprise a secondary interaction-compartment that is interconnected with the second compartment, and the method may in this case prior to the step of monitoring further comprise the steps of moving the at least one particle by means of the field into the secondary interaction-compartment of the system, and allowing the at least one particle to interact with a liquid contained in
25   the secondary interaction-compartment so as to make the result of the interaction between the reagents and the content of the liquid sample detectable by the detection means.

Likewise, and also according to the invention, the system may further comprise a
30   washing-compartment that is interconnected with any of the other compartments, and the method may in this case further comprise the steps of moving the at least one particle into the washing-compartment of the system by means of the field, and

allowing the at least one particle to interact with a liquid contained in the washing-compartment so as to remove unwanted material from the at least one particle.

The device according to the invention may comprise any combination of one or

5   more of the above-mentioned types of compartments.

One of the compartments of the system according to the invention may optionally be adapted for letting electromagnetic radiation of certain wavelengths reach the liquid contained in said compartment for enabling a process of photoactivation to

10   take place. The method may in this case further comprise the step of subjecting the at least one particle to electromagnetic radiation of a wavelength suitable for causing a process of photoactivation.

The at least one particle that is used in the above described devices and methods

15   may in a preferred embodiment of the invention be of a mean diameter of 1-200 micro meter, more preferred of 1-50 micro meter and most preferred of 2-20 micro meter.

The cross-sectional dimensions of said compartments may in a preferred

20   embodiment of the invention of the above described devices and methods be within 100-1000 micro meter, more preferred within 100-600 micro meter and most preferred within 200-500 micro meter, but at least one of the cross-sectional dimensions may also be in a smaller range, within 35-100 micro meter.

25   The device according to the invention is preferably manufactured from materials that are non-magnetic and/or from materials that are non-autofluorescent, such as Topas.

The compartments are preferably made from materials that do not interfere

30   significantly with the function of the compartment. The compartment materials should not alter the contents of the compartment and not be altered itself by the compartment contents. Typical compartment material could be polymers (milled, injection moulded etc.), glass, ceramics.

The interconnections between compartments in the device comprising the system are in some embodiments of the invention closed until they by activation are opened prior to the performance of a test so as to prevent the contents of the compartments to mix before a test.

5

The interconnections may be closed with a material that is solid before activation, and the activation is performed by heating at least a part of the system of compartments until the closing material becomes liquid.

10

Alternatively, the activation of the interconnections may be performed by physically aligning the compartments.

Additional features such as electrodes for heating and temperature measurements and light guides for entering light into a compartment and getting light out from the compartment may be included.

15

Besides reagents the compartments may contain a wide range of additive. As an example and not limitation of the function of these additives one could mention pH-stabilisation, adjustment of viscosity, preservation (e.g. against microorganisms), adjustment of surface tension.

20

The invention further concerns a method of analysing the content of a liquid contained in a container, the method comprising the steps of

25

(a) mixing particles with the liquid so as to obtain a substantially even distribution of the particles in at least a part of the liquid, the particles being at least partly made from a material susceptible to a field, such as a magnetic field or an electric field, and having at least one reagent immobilised on a plurality of the particles,

30

(b) allowing the reagent immobilised on the particles to interact with the content of the liquid,

(c) applying a field to which the particles are susceptible to at least a part of the container so as to move at least one of the particles through an opening of the container to extract the at least one particle from the container,

5

(d) moving the at least one particle through a liquid-filled passage to detection means for detecting properties of the reagents on said at least one particle, and

10 (e) detecting properties of the reagent on said at least one extracted particle in order to determine whether these properties have changed due to the interaction, so as to perform an analysis of the liquid.

The steps (c) to (e) may be repeated at least once after elapse of a predetermined
15 time period so as to provide a monitoring of a possible ongoing process involving the liquid.

The invention also concerns a system for distinguishing between particles with different magnetic properties, the system comprising
20

first detection means, such as a Hall sensor, for detection of the magnetic properties of particles and adapted to provide an output according to the magnetic properties of a particle comprised within a first measuring volume of the first detection means,
25

a population of particles made at least partly from a material with magnetic properties that are detectable by the first detection means, the population of particles comprising at least two subpopulations of particles, each subpopulation of particles having different magnetic properties so that said output from the first
30 detection means may provide a significant indication of which subpopulation a detected particle is a member of,

a liquid in which the particles are contained when the magnetic properties of the particles are being detected by the first detection means, and

5   a member with a flow channel defined therein for leading the liquid with the particles contained therein through the first measuring volume, the flow of the liquid being controlled in such a way that one particle at a time passes the first measuring volume.

The particles comprised in the system for distinguishing between particles with
10   different magnetic properties may have surface properties suitable for immobilising at least one reagent thereon and have reagents immobilised on a substantial number of the particles within each subpopulation so that each subpopulation has a specific reagent assign to it and at least two of the subpopulations have different specific reagents assigned to them, so as to enable performance of an analysis with
15   at least two different specific reagents of the content of a liquid sample.

The system for distinguishing between particles with different magnetic properties may preferably comprise second detection means for detecting properties of reagents immobilised on particles comprised within a second measuring volume, for
20   determining whether said properties have changed during an interaction between the content of the liquid sample and the particles, so as to perform an analysis of the content of the liquid sample. Preferably, the second measuring volume is positioned and the flow of the liquid with the particles contained therein is controlled in such a way that particles passing the first measuring volume one at a time also
25   passes the second measuring volume one at a time.

For controlling the flow in such a way that particles pass the first and optionally the second measuring volume one particle at a time, the flow channel defined in the member may optionally comprise means for entering at least one buffer-liquid into
30   the flow channel parallel to the flow of the liquid with the particles contained therein.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 shows the forces acting on particles subjected to a magnetic field,

5   Fig. 2 shows a chip with an H-shaped channel therein,

Fig. 3 shows the H-shaped channel with adjacent magnets,

Fig. 4 is a diagram of an experimental setup including the chip with the H-shaped
10   channel,

Fig. 5 and Fig. 6 A-D shows different stages of one experiment performed using the
chip with the H-shaped channel,

15   Fig. 7, Fig. 8 and Fig. 9 shows expected results and actual results from the
experiment performed using the chip with the H-shaped channel,

Fig. 10a illustrates competitive affinity assays,

20   Fig. 10b illustrates non-competitive affinity assays,

Fig. 11 shows the chemical principle of the IgG-assay,

Fig. 12a shows a linear compartment arrangement,

25
Fig. 12b shows five linear compartment arrangements placed in parallel,

Fig. 13 shows a compartment arrangement including a bend,

30   Fig. 14 shows a linear compartment arrangement comprising a number of magnets
for letting the particles describe a zigzag path,

Fig. 15 shows an embodiment of a system adapted for performing light induced DNA synthesis,

Fig. 16 describes a process for synthesising oligomers of DNA,

5

Fig. 17 shows a setup for measuring the MRM-content in particles,

Fig. 18 shows a device for magnetic sampling and illustrates the method.

10 Fig. 19 shows the principle of magnetic sampling without suspending the particles in the sample.

Fig. 20 is a schematic representation of the different types of convection that are discussed.

15

Fig. 21.A is an exploded view of the prototype chip used for the interaction experiment.

Fig. 21.B is a view of the interaction chip as used.

20

Fig. 22.A shows a cross sectional view of the contents of the compartment complex before the interaction experiment was performed.

Fig. 22.B shows a cross sectional view of the contents of the compartment complex
25 while the particles move through the sample compartment.

Fig. 22.C shows a cross sectional view of the contents of the compartment complex while the particles move through the washing compartment.

30 Fig. 23 shows results from the interaction experiment: The responses at different concentrations of analyte.

Fig. 24 shows a disposable chip containing a multitude of parallel micro systems placed in a reusable apparatus.

5

DETAILED DESCRIPTION OF THE FIGURES

The figures are described in more detail herein below. The objective of the figures is to illustrate the invention and the figures should be interpreted as merely

10 presenting preferred embodiments of the present invention.

Forces on particles

15 Fig. 1 shows the forces that act on magnetic particles 1 in a liquid system. F_m is the magnetic forces between the particles and the magnet 2, F_f is the friction force from the liquid acting on the moving particles 1, F_g is the gravitational force on the particles 1 and F_{ip} is the magnetic attraction between particles 1. The particles 1 are assumed to be out of contact with the walls that confine the liquid system. However,

20 this assumption may not be valid for all cases that may be considered in connection with the invention. We assume liquid is ideal. We assume the Stoke law applies to a particle moving in the fluid. Deviations from this should not be limiting for the scope of the protection of the present invention.

25

Horizontal movement

The movement of a particle 1 towards the magnet 2 due to attraction between the magnet 2 and the particle 1 is called horizontal movement. The two forces involved

30 in that movement are the magnetic force F_m (between the magnet 2 and the particle 1) and the friction force F_f (friction between the particle 1 and liquid).

The equation that governs the magnetic force on a single particle 1 is given as

$$\vec{F}_m = \frac{1}{2} \cdot N_{fer} \cdot \frac{V_{fer} \cdot \Delta \chi_{fer}}{\mu_0} \cdot \nabla \vec{B}^2$$

where N_{fer} is the number of magnetite pieces in a particle 1, V_{fer} is the mean volume of the magnetite pieces and χ_{fer} is the net magnetic susceptibility of magnetite (χ_{fer}-

5   χ_{liquid}). Furthermore, μ_0 is the vacuum permeability and ∇B^2 is the gradient of the square of magnetic field of the magnet 2.

The friction force F_f acting on particles 1 where the Reynolds number for the movement of the particle 1 relatively to the surrounding fluid is less than 0.2, may

10 be found from Stokes formula which gives the viscous drag force or friction force of a spherical particle 1 in a liquid as

$$\vec{F}_f = 6 \cdot \pi \cdot R \cdot \eta \cdot \Delta \vec{v}$$

where R is the radius of the particle 1, η is the viscosity given in Poise and Δv is particles speed relative to the liquid.

15

The particles 1 may for most relevant situations be assumed to be in a quasi steady state so that

$$\vec{F}_m = \vec{F}_f$$

By substituting F_m and F_f with their equivalent expressions the following equation is

20 achieved

$$\frac{1}{2} \cdot N_{fer} \cdot \frac{V_{fer} \cdot \Delta \chi_{fer}}{\mu_0} \cdot \nabla \vec{B}^2 = 6 \cdot \pi \cdot R \cdot \eta \cdot \Delta \vec{v}$$

from which the velocity of the particle 1 relatively to the fluid easily is isolated

$$\Delta \bar{v} = \frac{1}{12} \cdot N_{fer} \cdot \frac{V_{fer} \cdot \Delta \chi_{fer}}{\mu_0 \cdot \pi \cdot R \cdot \eta} \cdot \nabla \bar{B}^2$$

Parasitic movements

5　Movements of the particles 1 that are unwanted for the analytical purpose are called parasitic movements. Examples of these are gravitational settling of the particles 1, magnetic attraction between particles 1 and convection in the liquid, although these movements are wanted in some situations, e.g. where particles are moved by the gravitational force. Only gravitational settling is dealt with in the

10　following.

Two forces are involved in gravitational settling. One is the force of the gravity acting on a particle 1 suspended in a liquid and the other is the friction between the particle 1 and the liquid.

15

According to Archimedes' equation, the net gravitational force acting on a particle 1 suspended in liquid is

$$\bar{F}_g = (\rho_p - \rho) \cdot V_p \cdot \bar{g}$$

where ρ_p and ρ are the densities of the particle 1 and the liquid, respectively, V_p is

20　the volume of the particle 1 and g is gravitational acceleration.

The steady-state velocity of settling particles 1 can be calculated from the equality

$$\bar{F}_g = \bar{F}_f$$

When the forces are replaced by their formulas this gives

$$\Delta \bar{v} = \frac{(\rho_p - \rho) \cdot V_p \cdot \bar{g}}{6 \cdot \pi \cdot R \cdot \eta}$$

As the volume of a spherical particle 1 is given as $V_p = 4/3 \cdot \pi \cdot R^3$, the settling velocity can be expressed as

$$\Delta \bar{v} = \frac{(\rho_p - \rho) \cdot \frac{2}{9} \cdot R^2 \cdot \bar{g}}{\eta}$$

5   A first example of settling is given for Dynal M-280 particles 1 in a phosphate buffer. The particles 1 of a diameter of 2.8 μm and a density of 1300 kg/m3 are suspended in water with a density of 1000 kg/m^3 and viscosity of 1 cP (10^{-3} kg/(m-1s-1)). This gives a settling velocity of

$$\Delta \bar{v} = 1.3 \cdot 10^{-6} \, m/s$$

10   A second example of settling is given for Dynal M-280 particles 1 in a sucrose buffer with a density of 1240 kg/m^3 and a viscosity of around 30 cP. The settling velocity is in this case found to be

$$\Delta \bar{v} = 0.0086 \cdot 10^{-6} \, m/s$$

The gravitational settling may be reduced by counteracting the gravitational force
15   with e.g. a magnet placed above a channel in which magnetic particles are situated.

A system that has been used for the initial tests of the magnetic particle handling is shown in Figs. 2-9 together with the results of the test. Fig. 2 shows the flow chip 3 that was used, comprising a top part 4 (8*40*15 mm) and a lid 5 (2*40*15 mm) both
20   made from PMMA. An H-shaped channel 6 was milled in the surface of the top part 4. The channel 6 has a depth of 400 μm and a width of 400 μm. The H-shaped channel 6 is 30 mm long, and the interconnection line 7 is 5 mm wide. 1.4 mm diameter holes 8 were drilled at each of the four ends of the H. A piece of tubing, 1/16 inch outer diameter and 0.8 mm inner diameter, was inserted in each hole 8 so
25   as to form a liquid interface from the exterior to the channel 6. The milled surface of the top part 4 was wetted with acetone and pressed together with the lid 5. The

combined of top part 4 and lid 5 is shown in Fig. 2 on the right. A fluorescence microscope 9 equipped with a photo multiplier tube for light detection was provided for monitoring of the interior of the channel.

5   Fig. 3 shows the positioning of the magnets relative to the liquid filled channels 6. The primary magnets 10 are optionally placed in such a way that they may provide a force by which the particles 1 may be moved back and forth in the interconnection channel 7. Preferably, electromagnets 10 are used, comprising an iron core with length 80 mm and diameter 6 mm and about 400 windings of a 0.5 mm outer

10   diameter copper wire. At the end surface of the electromagnets 10 a magnetic flux density of at least 0.22 Tesla (at a voltage of 3.1V and a current of 3.3A) may be achieved. The primary magnets 10 are placed as close as possible to the interconnection channel 7 of the flow chip 3 to optimize the effect from the magnets 10 on the particles 1.

15

A secondary magnet 11 is provided as a counter measure to prevent the particles 1 from settling and is a Samarium Cobalt permanent magnet 11 (10*6*30 mm) from Goudsmit, The Netherlands. The force from the secondary magnet 11 on the 1 particles is in a direction opposite to the force of gravity. The magnetic flux density

20   is 0.5 Tesla at the surface of the permanent magnet 11. The secondary magnet 11 is placed approximately 25 mm above the H-shaped channel 6 to achieve the reduction in settling of particles 1.

To automate the filling of compartments and the assay procedure, a computer

25   controlled flow injection system was set up. Fig. 4 shows a schematic view of the system including the flow chip 3. The full lines 12 indicate PFTE teflon tubing of outer diameter of 1/16 inch and inner diameter of 0.8 mm for providing liquid connection between the different components, together with suitable fittings. The dotted lines 13 indicate data communication means for providing data

30   communication between the Personal Computer 14 and various components. Two step motor driven syringe pumps 15, 16 were used for driving liquid through the H-shaped channel 6. Three three-way valves 17 are used for precise liquid control, such as for filling one of the pumps 15 with buffer from the buffer reservoir 18, for

aspiration of particle suspension from the suspension vial 19 and for addressing the individual outlets of the flow chip 3.

The magnets 10 (only one is shown) and the detection system 9 are also indicated in Fig. 4. All the active components are linked to a Personal Computer 14 that is equipped with a multifunction card. The multifunction card and the control software is from National Instruments, USA.

The particles used in the present experiment are from Dynal AS, Norway. The particles have a density around 1.3 g/mL and are superparamagnetic. They have streptavidin immobilised on their surface, which allows for coupling with biotin-bound reagents and aminoreactive dyes.

The particles were labelled with fluorescein isothiocyanate (FITC) according the following prescription: 10 mg FITC was brought into solution in 1 mL dimethylsulfoxide. 1000 µL magnetic particle suspension was transferred to a dark duran glass flask and added 10 mL pH 9.5 carbonate buffer (0.1 M) and 500 µL FITC-solution. The mixture was shaken well and allowed to react for 12 hours at room temperature. Unbound FITC/fluorescein was removed from the labelled particles by successively washing and separating the particles magnetically from the washing liquid until no more background fluorescence from the liquid was detected. A pH 7.4 phosphate (0.1 M) buffered saline solution was employed both for the washing process and as an inert buffer for the characterisation. For the final experiments the effective dilution of the manufactures stock solution was 1/330 giving 0.03 mg particles/mL.

A more detailed picture of the compartment complex used for the characterisation experiments is shown in Fig. 5. The H-shaped channel 6 comprises the compartment for particle supply 20, the compartment for detection 21 of properties of the particles and the compartment for sample interaction 7. The two primary magnets 22, 23 are placed outside the H-shaped channel 6 in such a way that the steepest gradient of the magnetic field goes through the compartment for sample interaction 7. The compartment for particle supply 20 can be replenished by

sending in a suspension of particles at the inlet 24 and let the used suspension leave the compartment at the outlet 25. The inlets 24, 26 and outlets 25, 27 can be addressed individually through the use of external valves (not shown in the figure), which allows for flexible filling of the compartments.

5

The particles 1 in suspension have higher density than the liquid in which they are suspended but the buoyancy force is partly cancelled out by the secondary magnet 11. The compartment for sample interaction 7 is in the characterisation experiments filled with a buffer that does not alter the fluorescent properties of the beads.

10

The detection window 28 in the compartment for detection 21 is represented as a black rectangular box in Fig. 5. The actual shape of the detection window 28 is defined by a pinhole in front of the photomultiplier of the fluorescence microscope.

15   The procedure of running one experiment is described in Fig. 6 A-D). The compartments are first filled with the liquid that they are supposed to contain. The detection compartment 21 and compartment for sample interaction 7 are filled with phosphate buffer and subsequently the compartment for particle supply 20 is filled with a suspension of particles 1. Fig. 6 A) illustrates the condition where all the
20   compartments are replenished.

The first magnetic action is to align the particles 1 in the compartment for particle supply 20. This is accomplished by turning on the primary magnet 22 closest to the supply compartment 20. Within less than 15 seconds the particles 1 are aligned as
25   shown in Fig. 6 B). The next step is to turn off the aligning magnet 22 and to turn on the primary magnet 23 that will pull the particles 1 through to compartment for sample interaction 7, this step is called the launch. Bigger particles and aggregates of particles 29 will move faster that single and smaller particles 1 which is also shown in Fig. 6 D). When the particles 1 have passed the detection window 28 and
30   the measurements have been made, the compartments 7, 20, 21 may be filled up again and a new analysis or synthesis can be performed.

The expected result of the experiment is shown in Fig. 7. The arrival of a fluorescent particle 1 to the detection window 28 and the measuring volume of the detector 9 results in a peak. Both the time of flight, which is the time from launch to the arrival at the detector, and the peak width are important fingerprints of the

5   identity of the particle 1. The first particles 1 to cross the detection window will be the biggest since bigger particles 1 move faster than smaller ones. The bigger particles 1 will move faster through the detection area than smaller ones due to their higher speed, thus making narrower peaks.

10   In the experiment with inert buffer in the compartment for sample interaction 7, the measured response from a single particle 1 will be independent of its time of flight. For the intended use, an interaction may take place between molecules in the sample placed in the compartment for sample interaction 7 and one or more reagents immobilised on the particles 1. In the case of slow kinetics of these

15   interactions, the time of flight will be highly correlated with the change in response of the particles 1. If each peak is plotted as peak height versus the time of flight, a pattern similar to the one shown in Fig. 8 would be expected. The measured responses that fall within the marked diagonal area are considered credible as they are the product of a controlled magnetic manipulation. Measurements that fall

20   outside the marked area are called forbidden as the response has reached the detector 7 in an uncontrolled manner. The credible peaks should be given the most weight in the data analysis. Only peaks within the solid black parts of the marked diagonal would be expected if an experiment where only single particles and aggregates of two particles are present is considered.

25

A plot of measured responses of an actual characterisation experiment is shown in Fig. 9. The plot in Fig. 9 reveals a clear tendency in the measurements towards that the narrowest and highest peaks, which are significant for the aggregates, are detected first and the broader and lower ones, indicating single particles, are

30   detected later.

Affinity assays - an immunoassay

The affinity assay is one of the working horses of analytical biochemistry. The affinity assay uses pairs of molecules that bind together with high strength, affinity.

5   Such pairs are antibody-antigen, avidin-biotin, chelate-metal, enzyme-inhibitor, DNA-DNA, RNA-RNA, etc. The reagent part of the affinity pair is often immobilised on a solid phase and the analyte part is sought in a liquid sample.

The solid phase is in the present case the particles 1, e.g. susceptible to magnetic

10   fields, and the analytes are captured by the reagent immobilised on the particles by letting the magnetic particles move through the sample. The affinity assays are typically performed in one of two modes named competitive and non-competitive assays. The chemical principle of each mode will briefly be explained in the following together with a description of two embodiments of the invention.

15

Competitive affinity assays

Labelled and unlabelled analyte are competing about affinity ligands immobilised on a solid phase in the competitive affinity assay of which the principle is shown in Fig.

20   10a. The solid phase 30 has been coated with the affinity ligand 31 and is surrounded by a liquid media containing unlabelled analyte 32, which is native to the sample and is present in unknown amounts, and labelled analyte 33 that is added in known amounts. An important feature is that the amount of labelled analyte 33 should be in excess compared to the amount of affinity ligand 31

25   immobilised on the solid phase 30. The solid phase 30 is washed with an in washing buffer after a certain reaction time and the remaining response from the labels on solid phase is measured with detection means, such as an optical fluorescence sensor. The ratio of labelled 33 and unlabelled analyte 32 on the solid phase 30 should the same as the ratio in the sample when the labelled analyte is

30   added, assuming similar reaction kinetics for the labelled and unlabelled analyte. The amount of analyte 32 native to the sample can be calculated since the amount of labelled analyte 33 is known.

The labels used for labelling an analyte 33 should be easy to detect with appropriate detection means and be of high sensitivity. Fluorescing probes like fluorescein, rhodamine and Texas Red are often used as labels. These probes exist in derivatives that are easily coupled to common analytes. Another type of label that

5   is often used is enzymes. The enzymes are catalytic molecules and the presence and concentration is detected by the amount of signal yielding reaction they cause, and enzyme labels are rather sensitive due to their signal amplifying abilities.

Non-competitive affinity assays

10

Two types of affinity ligands are employed in the non-competitive affinity assay as illustrated in Fig. 10b. The primary ligand 34 is immobilised on the solid phase 30 as was the case for the competitive assay. An amount of analyte 32 from the sample and secondary, labelled affinity ligands 35 that also bind to the analyte 32

15   are present in the surrounding liquid. The amounts of primary and secondary antibodies have to be in excess compared to the amount of analyte 32.

The analytes 32 will be bound to the solid phase 30 via the primary affinity ligand 34 and each bound analyte 32 will be attached a labelled secondary affinity ligand 35.

20   The solid phase 30 is after some reaction time washed with a suitable buffer and the response from the solid phase 30 is measured with detection means, such as an optical fluorescence sensor. The measured response is proportional to the amount of analyte 32 captured by the solid phase 30 whereas the response from the competitive assay was proportional to the ratio between labelled 33 and

25   unlabelled 32 analyte. This fact renders the non-competitive assay more sensitive than the competitive one.

One special application of the non-competitive affinity assay is making the diagnosis of viral infection (HIV, Epstein-Barr virus, influenza, etc.). To indicate the

30   presence of the virus in a person, a blood sample is taken and analyzed for antibodies (immunoglobulin G, IgG) against the specific virus. Fig. 11 shows the chemical principle of the IgG-assay. Particles 1, e.g. susceptible to a magnetic field, have been coated with surface groups 36 from the given virus. The IgG's 37 from

the blood sample - specific to the virus - will normally bind to the viral surface groups 36 and therefore attach themselves to the particles 1. To make the presence of IgG's 37 detectable, secondary affinity reagents 35 may be employed. They could be protein A (from Staphylococcus aureus) labelled with a fluorescent

5   label which could be fluorescein-isothiocyanate (FITC). When all the component have had time to interact, the particles 1 have become fluorescent due to the protein A-FITC that have bound to the virus specific IgG's 37 that have bound to the viral surface groups 36 on the particles 1. The fluorescence from the particles 1 is then detected.

10

Affinity assay embodiment

The procedures for making an affinity assay may be implemented in several ways
15   employing magnetic particle manipulation. Two preferred embodiments will be described in the following.

The first system shown in Fig. 12a is based on a linear compartment arrangement, where the compartment for particle storage 38 is followed by the compartment for
20   sample interaction 39 and finally the compartment for secondary reactions and/or for washing 40 comprising the measurement area 41 for detection of properties of the particles 1.

The secondary reaction and/or washing compartment 40 may when used for
25   competitive assays analysis be filled with a liquid for washing or a liquid containing labelled analyte. When used for a non-competitive assay analysis, the secondary reaction compartment 40 may be filled with a liquid containing the secondary, labelled affinity ligand.

30   An electromagnet 42 is placed at the right end of the linear complex of compartments to move the magnetic particles 1 coated with the primary affinity ligand through the compartments.

The complex of compartments sketched in Fig. 12a may be used as a disposable one-shot system, with magnetic particles 1 and secondary chemicals pre-filled into the compartments. The electromagnet 42 and the detection system are physically separated from the disposable compartment complex and can be used many times.

5   The only liquid that should be injected during operation of the complex of compartments for performing a test is the sample. Therefore, the only openings for passing liquid between the complex of compartments and the exterior that are open after the pre-filling of the compartments are the ones in the compartment for sample interaction 39 which has a sample inlet 43 and a sample outlet 44.

10

The complex of compartments may be made from a polymer e.g. by injection moulding or conventional milling. The dimensions of the shown embodiment are a compartment cross section of 400 μm times 400 μm, the length of the particle storage compartment 38 is 500 μm, the length of the compartment for sample

15   interaction 39 is 5000 μm and the length of the washing/detection compartment 40 is 3000 μm.

The linear compartment complex as the one shown in Fig. 12a has the advantage that a number of complexes may easily be placed in parallel as shown in Fig. 12b.

20   Here, five linear compartment complexes are arranged in parallel with respect too the direction of movement of the particles 1. The sample interaction compartments 39 of the compartment complexes are connected in series and are supplied with sample from a common sample inlet 43. Each linear compartment complex is adapted for measuring with a specific analyte or to make more tests for the same

25   analyte in order to enhance precision and to provide a validation for the results of the measurements.

If the involved affinity ligands are antibodies one could use magnetic particles 1 coated with protein A or G (which bind most IgG antibodies in a favourable

30   manner), streptavidin or biotin (e.g. streptavidin on the particles and biotin coupled to the antibodies) or reactive leaving groups as tresyl chloride or tosyl chloride. Such particles can be purchased from Bangs Laboratories (US) and Dynal (Norway).

Compartment complexes with the same function (e.g. affinity assays) may be arranged together in many ways. Fig. 13 shows a sketch of an alternative arrangement for the affinity assay. If longer compartment lengths are needed

5   without making the electromagnets stronger a bend between the sample interaction compartment 39 and the compartment for secondary reactions and/or for washing 40 may be made. The complex of compartments comprises in this case two magnets 45, 46, one magnet 45 for moving the particles 1 from the particle storage compartment 38 and through the compartment for sample interaction 39, and one

10   magnet 46 for moving the particles 1 through the compartment for secondary reaction 40 and into the measurement area 41. The effect of increasing the length of the sample interaction compartment 39 is that the particles 1 will interact with more sample when passing through the compartment 39 with means that the particle 1 have opportunity to interact with more analyte and the analysis thus

15   becomes more sensitive.

There are alternative ways of generating the magnetic gradients. As shown in Fig. 14 several magnets 47, preferably electromagnets, AC or DC, may be positioned along both sides of the linear compartment complex. Each magnet 47 may be

20   actuated individually and if the actuation is timed well the magnetic particles 1 will move following a zigzag path through the complex. In this way, the particles 1 are allowed to interact with more of the contents of the liquids in the system than if the particles 1 followed a straight path through the complex, thus enhancing the sensitivity of the analysis.

25

DNA synthesis

The synthesis of short strands of DNA is of importance when dealing with genetic analysis. Typical applications include screening for pathogenic micro organisms

30   and diagnosing genetic disorder. Short nucleotide probes can also be used as synthetic antibodies, which is very interesting since DNA is more stabile than protein. This means that short nucleotide probes may act as generic affinity ligands.

Light induced DNA synthesis

DNA is a polymer consisting of 4 different monomer units: adenosine (A), thymine
(T), cytosine (C) and guanine (G). The monomer units can be randomly combined
5   giving the single DNA strand different geometric shapes and genetic coding.
The process of synthesising oligomers of DNA by the means of controlled light
activation is well established (reference: 'The efficiency of Light -Directed Synthesis
of DNA-arrays on Glass Substrates', McGall et al, J. Am. Chem. Soc., 119 (22),
1997, p. 5081-5090) and even used in some commercial systems (Affymetrix,
10   USA).

A process for synthesising oligomers of DNA is described in Fig. 16. The magnetic
particles 1 are coated with a light sensitive molecule 48, as shown in Fig. 16 A.,
with an oxygen bridge that dissociates upon exposure to light of a certain
15   wavelength as shown in Fig. 16 B-C. When the particles 1 are moved into a
compartment with preactivated nucleotides, X-© 49, the nucleotides 49 will bind to
the activated sites 48 of the particle 1 and retain the ability to be activated itself as
shown in Fig. 16 D-E. The "X" of the nucleotides 49 symbolises that the compartment
may have been filled with any of the four nucleotides. By repeating the steps in Fig. 16
20   A-E four time and switching between compartments of thymine (T), cytosine (C),
adenosine (A), and thymine (T), one could obtain the sequence: T-C-A-T as shown in
Fig. 16 F.

An embodiment according to the invention of a system adapted for performing light
25   induced DNA synthesis is presented in Fig. 15. Four compartments 50-53 for
nucleotide-particle interaction (CNPI) are each filled with a pure solution of one of
the four nucleotides so that each nucleotide is represented. The four CNPI's are
combined with washing compartments 54 and an activation compartment 55 in
such a way that a magnetic particle 1 can travel between the compartments 50-55
30   in any order that should be desired. Four electromagnets 56 are positioned so that
they may move the particles between the compartments 50-53 containing the
CNPI's by their magnetic fields. Additionally, magnets could be included to reduce
settling effects from the gravitational field.

The CNPI's are sketched with inlets 57 and outlets 58 in order to allow for replenishing of the liquid in the compartments 50-55. The complex could also be made for one-shot purposes without allowing replenishment. The light activation
5   compartment 55 should allow light of the wavelength of activation to reach the interior of the compartment 55, e.g. by having a top or bottom wall that is transparent to the wavelength of activation.

The true force of the method of magnetic particle 1 manipulation is unleashed when
10   synthesis and analysis is combined in a kit. The synthesis of DNA affinity ligands may preferably be computer controlled, thus the result of the synthesis may be determined by the software/user and not the reagents initially immobilised on the beads. Therefore, the user may choose to analyse for any analyte that can be bound by a DNA-oligomer using the same system, since the affinity reagents are
15   generated just before the analysis. This arrangement allows for great flexibility and applicability of a single system.

Magnetic barcoding

20   The principle of magnetic bar coding is to identify individual particles as belonging to a specific subpopulation of particles among a number of subpopulations by detecting the magnetic properties of the particles 1, e.g. the amount of magnetically responsive material (MRM) in the particles 1. The particle 1 will, depending on its MRM loading, create a distortion of an external magnetic field and this distortion
25   may be measured with a Hall-sensor that may be either integrated in the chip with the flow channel or be external.

The particles 1 are divided in subpopulations based on the MRM-loading (or similar loading per volume and different size). The differences in MRM loading (or size)
30   between the subpopulations should be big enough to distinguish between them with the Hall-sensor. It is possible to achieve a high number of subpopulations if desired, at least 50-100 and up to 200 groups with some effort. The two mayor applications

of this aspect of the invention are particle based multi analyte assays and test of small combinatorial libraries.

Multi analyte assays

5

For the multi analyte assay one needs a population of particles 1 consisting of many subpopulations. Each subpopulation of particles is to be coated with a certain reagent. The subpopulations are mixed into one population and exposed to the sample. If analytes are present the in sample the magnetic particles with the

10   corresponding reagent will change their response. By looking at the particles separately both their magnetic content and analytical response can be measured.

A possible setup for measuring the MRM-content in the particles is shown in Fig. 17. A suspension of particles 1 that have been incubated with a sample is

15   hydrodynamically focused by sending it through a center flow channel 59 and sandwich it between two streams of inert buffer 60. In this way, the particles 1 are tightly positioned in the measurement channel 61 so that the particles 1 passes a cross-section of the measurement channel 61 one at a time. A magnet 62, e.g. a permanent magnet or an electro magnet, is placed so that its field penetrates the

20   measurement channel 61 and a Hall-sensor 63 is positioned on the opposite side of the channel 61. The Hall-sensor 63 measures the flux density of the magnetic field and detects changes in the field caused by the magnetic particles 1. Besides the Hall-sensor 63 an optical measuring window 64 is placed in the channel to make it possible to correlate the magnetic properties measured by the Hall-sensor 63 to the

25   optical measured properties for each specific particle 1. For example, fluorescence may be measured from the particles through the optical measuring window 64 with a fluorescence detector, such as a photomultiplier or a CCD array arranged with a suitable objective.

30   Optionally, two pieces 65 of soft magnetic material (e.g. a soft ferromagnetic material) may be inserted on each side of the measurement channel 61 to focus the magnetic field so that the measurement volume of the Hall-sensor 63 is decreased and the spatial resolution thereby is increased.

Magnetic sampling

Sampling in analytical chemistry covers the operation of taking out a representative subpart of the entire liquid (or solid or gas) that is to be analyzed. Representative
5   sampling is a far-from-trivial problem that is often not a part of automated analysis systems. A common approach is to take out a liquid sample from one point and assume that the composition of the remaining liquid is similar to the sample.

The novelty of the proposed method according to the invention, magnetic sampling,
10   is to probe the entire liquid with magnetic particles coated with analyte specific reagents, instead of taking out and analysing a small subpart of the sample, so-called subpart sampling. Reagent coated magnetic particles 1 are suspended in the medium that is to be analyzed, see Fig. 18, where the liquid in the shown example is contained in a stirred liquid container 66. The container 66 could for example be
15   a fermentation tank for biotechnological production, a waste-water treatment plant or lake. The magnetic particles are, when a magnet 67 is activated, moved from the liquid to be analyzed to the analysis system through a liquid connection between the container 66 and the analysis system.

20   Conventional techniques/methods known in the art of chemical analysis (e.g. flow injection with fluorometry or absorptivity) may be used in the analysis system.

An advantage of the magnetic sampling compared to subpart sampling is that the probe particles 1 can preconcentrate the analyte of interest on their surface.
25   Therefore, the surface concentration of analyte on the particles 1 will be magnitudes higher than the concentration in the entire liquid to be analyzed, and the upcoming analysis will have an increased sensitivity to the analyte.

Furthermore, magnetic sampling is more robust than subpart sampling. Sucking out
30   a heterogeneous liquid sample may lead to clogging in the analysis flow system followed by malfunction. For the analysis magnetic sampling only takes in the well-defined probe particles. Therefore the risk of clogging and system contamination is significantly reduced.

49

When no more analysis are required, the magnetic probe particles are easily removed from the main liquid with a magnetic field.

5   Another new feature is sequentially to collect the particles 1 during the time of monitoring. This is done by applying a magnetic field by activating the magnet 67 and then analyse the particles 1 as soon as they enter the analysis system. This will give a timely resolution of the measured parameters in the liquid to be analyzed and is usable for chemical process control and environmental monitoring.

10

A third new feature is shown in Fig. 19 a-c. The reagent coated magnetic particles 1 are not suspended in the unknown liquid, but are moved to the interface 68 between the analysis systems 69 and the liquid to be analyzed 70 as shown in Fig. 19a and held there as shown in Fig. 19 b, where both the movement and the

15   holding is performed with magnetic forces. At the interface 68, the reagent coated magnetic particles 1 will interact with the liquid to be analyzed 70 for a controlled time period and will afterwards be moved back into the analysis system to complete the analysis as shown in Fig. 19c.

20   Diagnostic methods

The present invention also pertains to diagnostic methods. Preferred embodiments are use of the device according to the invention and/or the device for use in a diagnostic method, said method comprising the steps of contacting a sample, preferably

25   a biological sample, with a reagent immobilised onto a particle susceptible of field manipulation, said reagent being specific for a target substance putatively contained in said sample, isolating said reagent contacting said target substance, detecting said target substance either directly or indirectly, and optionally quantifying the presence of said target substance in said sample.

30

The diagnostic method according to the invention may be used in the diagnosis of any state-of-the-art biological substance, such as any biological organism including i) a mammalian cell, including human cells and eukaryotic cells, or a part thereof, ii)

a virus, including mammalian vira, human vira and eukaryotic vira, or a part thereof, and/or iii) a parasite, including mammalian parasites, human parasites and eukaryotic parasites, said cell, virus and/or parasite forming part of the human or animal body under normal physiological condition, or under conditions diagnosable as an

5   illness, a disease, a syndrome, a deficiency or any other potentially treatable, characterisable or diagnosable condition. Examples of cells, viruses and parasites are given herein below.

The method of diagnosis may also be directed to target substances such as e.g.

10   microbial organisms such as yeasts and fungi, slime molds and microorganisms in general and pathogenic microorganisms in particular. Examples of microorganisms are given herein below. Also in this case will the person skilled in the art know how to match these microbial target organisms with a reagent exhibiting specificity for said target organism.

15

The person skilled in the art will generally know how to use or formulate a reagent specific for any target substance of interest. The skilled person will also have at his disposal general and specialised medicine and biochemistry textbooks and reference collections.

20

Preferred reagents according to the invention include antigenic and/or immunogenic determinants including antibodies and monoclonal antibodies for the contacting of corresponding antigens and vice versa. This may be of prime interest when analysing an antibody response to a particular antigen or when screening a human be-

25   ing for a pathogen organism such as a potentially lethal virus or a pathogenic microorganism.

The method of diagnosis according to the present invention may be used e.g. in a diagnosis of severe combined immune deficiency, DiGeorge syndrome, MHC class

30   I deficiency, MHC class II deficiency, Wiskott-Aldrich syndrome, common variable immunodeficiency, X-linked agamma-globulinemia, X-linked hyper-IgM syndrome, selective IgA and/or IgG deficiency, phagocyte deficiencies, complement deficiencies, natural killer (NK) cell defect, X-linked lympho-proliferative syndrome, Ataxia

telangiectasia, and various autoimmune lympho-proliferative diseases. The skilled person will now how to select reagents capable of diagnosing directly or indirectly each of the above-mentioned conditions.

5   It may also be possible to detect, according to another presently preferred method of the invention, particular cellular changes and/or an altered cellular differentiation. As one example one can mention the altered number of CD4 cells during an infection of a human being with Human Immunodeficiency Virus (HIV). Many cellular and subcellular events may be monitored by examination of specific cellular determi-

10   nants, or CD antigens, expressed by a variety of cells including cortical thymocytes, Langerhans cells, dendritic cells, B cells and subsets thereof, including B cells in chronic lymphatic leukemia, T cells and activated T cells and precursors thereof, helper T cells and inflammatory T cells, cytotoxic T cells, thymocytes, monocytes, leukocytes, lymphocytes, macrophages, pluripotential hematopoietic cells, eosino-

15   phils, basophils, neutrophils, natural killer cells, platelets, brain and peripheral nerve cells, vascular smooth muscle cells, intestinal epithelium cells, smooth muscle cells, blood vessels, bone marrow stromal cells, myeloid cells, granulocytes, myelomono- cytic cells, and follicular dendritic cells.

20   It may also be possible according to another presently preferred method of the in- vention to detect or diagnose the presence of undesirable and pathogenic microor- ganisms such as e.g. microorganisms belonging to arts and species such as Achromobacter xylosoxidans, Acinetobacter calcoaceticus, Actinomyces israelii, Aeromonas hydrophilia, Alcaligenes, Arizona hinshawii, Bacillus anthracis, Bacillus

25   cereus, Bacteroides fragilis, Bacteroides melaninogenicus, Bordetella pertussis, Borrelia recurrentis, Brucella, Calymmatobacterium granulomatis, Campylobacter fetus ssp. Intestinalis, Campylobacter fetus ssp. Jejuni, Chlamydia, Chromobacte- rium violaceum, Citrobacter, Clostridium botulinum, Clostridium perfringens, Clos- tridium difficile, Clostridium tetani, Corynebacterium diphteriae, Corynebacterium,

30   Coxiella burnetti, Edwardsiella tarda, Eikenella corrodens, Enterobacter, Erysipelo- thrix rhusiopathiae, Escherichia coli, Flavobacterium meningosepticum, Francisella tularensis, Fusobacterium nucleatum, Gardnerella vaginalis, Haemophilus ducreyi, Haemophilus influenzae, Klebsiella pneumoniae, Legionella, Leptospira interro-

gans, Listeria monocytogenes, Moraxella, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia, Pasteurella multocida, Peptococcus magnus, Plesiomonas shigelloides, Proteus , Providencia, Pseudomonas aeruginosa, Pseudomonas

5   mallei, Pseudomonas pseudomallei, Rickettsia, Salmonella, Serratia, Shigella dysenteriae, Spirillum minor, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus, Streptobacillus moniliformis, Streptococcus, Streptococcus agalactiae, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema carateum, Treponema pallidum, Treponema pertenue, Ureaplasma urealyti-

10   cum, Vibrio cholerae, Vibrio parahaemolyticus, Yersinia enterocolitica, and Yersinia pestis

Examples of viruses the presence of which in a sample is detectable according to the method of the invention is Adenoviruses, Arena viruses, Astroviruses, Bunyavi-

15   ruses (Hantaan viruses), Cytomegalovirus, Calici viruses, Epstein-Barr virus, Filoviruses including Ebola virus, Hepatitis A virus, Hepatitis B virus, Hepatitis C virus, Herpesviruses, including Human Herpes Simplex viruses 1 and 2, Myxoviruses, Papillomaviruses, Papovaviruses, Parvoviruses, Picornaviruses, Togaviruses (Rubella virus), Paramyxoviruses and Orthomyxoviruses, Poliomyelitis Virus, Pox vi-

20   ruses, Reoviruses, Rhabdoviruses, Retroviruses, including Human Immunodeficiency Virus (HIV), Lymphadenopathy-Associated Virus (LAV), and Human T-lymphotropic Virus (HTLV), including any derivative thereof, including HTLV-I, HTLV-II, and HTLV-III.

25   By using the method according to the invention it is thus possible to detect any cell, virus or parasite being the cause of e.g. an infectious disease such as e.g. Acquired Immuno Deficiency Syndrome, Actinomycosis, Adenovirus infections, Anthrax, Bacillary dysentery, Botulism, Brucellosis, Candidiasis, Cellulitis, Chancroid, Cholera, Coccidioidomycosis, Common cold, Conjunctivitis, Cystitis, Dermatophytosis,

30   Diphtheria, Endocarditis, bacterial, Epiglottitis, Erysipelas, Erysipeloid, Gastroenteritis, Genital herpes, Glanders, Gonorrhea, Hepatitis, Histoplasmosis, Impetigo, Infectious mononucleosis, Influenza, Legionnaire's disease, Leprosy, Leptospirosis, Lyme disease, Melioidosis, Meningitis, Mumps, Nocardiosis, Nongonococcal ure-

thritis, Pinta, Plague, Pneumococcal pneumonia, Poliomyelitis, Primary atypical pneumonia, Pseudomembranous enterocolitis, Puerperal sepsis, Rabies, Relapsing fever, Retrovirus infections, Rheumatic fever, Rocky Mountain spotted fever, Rubella, Rubeola, Scarlet fever, Staphylococcal scalded skin syndrome, Streptococcal

5   pharyngitis, Syphilis, Tetanus, Toxic shock syndrome, Toxoplasmosis, Tuberculosis, Tularemia, Typhoid fever, Typhus, Vaginitis, Varicella, Warts, Whooping cough, · Yaws, and Yellow fever.

Consequently, having used the device and the method of detection and/or diagno-

10   sis according to the invention in order to detect an infectious agent known to be associated with any or more of the above, the skilled person will readily be able to perform a diagnosis and recommend a treatment, if any.

The skilled person will also know how to test the efficacy of a vaccine by testing in a

15   sample from an individual the antibody response of said individual following a vaccination. In this way, the efficacy of the following may readily be tested: Diphteria toxoid, Tetanus toxoid, Pertussis vaccine, Poliovirus vaccine, Rubeola vaccine, Mumps vaccine, Rubella vaccine, Influenza vaccine, Bacille Calmette-Guérin or BCG, Rabies vaccine, Typhoid vaccine, Cholera vaccine, and Yellow

20   fever vaccine.

EXAMPLE

25   The purpose of this example is to further demonstrate the feasibility of binding an analyte immobilised on reagent coated particles in a proto-type system according to the invention.

Experimental design

30

The compartments were made refillable by including inlets and outlets to make the prototype usable more than once. The invention was implemented in a reusable system where the refillable compartments were machined into PMMA (Poly Meth-

ylMethAcrylate). Figure 21.A shows how the system (called the chip) was build. The chip consisted of 3 blocks of plexiglass. The top block 77 was for liquid interconnection and for that purpose four holes 78 were drilled through the block (ID 1.4 mm). In the centre of the middle block 79 the compartment complex 80 was made

5   (a vertical hole that had been drilled all the way through the block, ID 0.5 mm). Channels (0.4 mm time 0.4 mm) had been milled both in the top 81 and the bottom 82 of the middle block as to provide inlets and outlets for sending in reagents in the compartments. Furthermore a hole 83 was drilled from the side to make it possible to fill in sample.

10

The bottom block 84 sealed the bottom channel and was used as a transparent lid. The optical detection was performed using a fluorescence microscope equipped for the fluorescent label Cy5 and with a photo multiplier for light detection.

15   PTFE tubings were fitted into the holes of the top block and the surfaces of the blocks were wetted with acetone. The acetone softens a thin layer of the polymer and when the blocks were pressed together they stuck like they were glued. A schematic drawing of the chip as used is found in Figure 21.B

20   All the liquid connections were hooked up to pumps and valves that allowed for a precise manipulation of liquid for filling the compartments. A special kind of on-off valve was used on each inlets/outlet of the chip. The special feature of the valve was that opened and closed without displacing any liquid, thus without disturbing the contents of the compartment complex.

25

Chemicals

The buffers used in the system were all based on 0.001 M HEPES and had a pH of 7.4. In the lower part (this is explained later) of the compartment complex a high

30   density buffer (15 % sucrose) was used and in the upper part the plain HEPES buffer was used.

The sample consisted of avidin-Cy5 in varying concentrations in HEPES buffer. Cy5 was a fluorescent label that is easily bound to proteins such as avidin. Avidin binds strongly to a molecule called biotin and to let the particles pick up avidin they were coated with BSA-biotin (Bovine Serum Albumin labelleb with biotin).

5

The particles (M500, Dynal, Norway) were bought with tosyl activation. In order to coat the particles with BSA-biotin, 100 μL of the particle suspension was washed in a pH 9.5 0.005M carbonate buffer using magnetic separation. 2.23 mg of BSA-biotin was dissolved in 0.5 mL pH 9.5 0.005M carbonate buffer and added to the

10 washed particles giving a total suspension volume of 1 mL. The mixture was incubated for 24 hours under gentle rotation at room temperature. After the incubation the particles were washed with pH 8 TRIS buffer and stored at 4°C in the TRIS buffer.

15 To prepare a particle suspension for the experiment 100 μL of the BSA-biotin coated suspension was washed with HEPES buffer and then resuspended in the HEPES buffer. The suspension was always mixed in a vortex mixer before a run was made.

20 <u>Procedure</u>

The procedure for liquid handling in an experiment is illustrated in Figure 22.A-C. Figure 22.A is a cross section of the middle block 79 and centred on the compartment complex 80. Prior the experiment the different reagents/samples have been

25 introduced. In the upper channel 81 a suspension of the biotin-BSA coated particles 1 have been introduced, the upper half of the vertical hole has been filled with the sample 85. The lower half of the system is filled with 15% sucrose buffer 86. All the valves surrounding the chip are shut off to create a system without convection. The focus 87 of the fluorescence microscope is placed on the part of the lid that is

30 touching the compartment complex to measure the fluorescence of all the particles that settle there.

The reagent-coated particles are heavier than the buffer they are suspended in and therefore the gravity will pull them downwards and slowly move through the sample compartment. During the contact with the sample the biotin of the particle surface will bind avidin-Cy5 from the sample (illustrated in Figure 22.B). After the sample

5   interaction the particles move into the buffer below the sample compartment and here loosely bound molecules will be washed away from the particle surface (illustrated in Figure 22.C). The particles still move downwards and settle the microscope focus on the lid where their fluorescence is measured.

10   Results

The result obtained from the experiments with the interaction chip and the chemicals described herein above is shown in Figure 23. It is clear that the concentration of avidin-Cy5 changes from 0 to 100 nM and the increasing response

15   from the particles is directly correlatable to the avidin-Cy5 concentration with a high degree of sensitivity. The response is approximately linear with the avidin-Cy 5 concentration.

20   Chip

A preferred embodiment of the invention deals with disposable chips with a least one micro system and preferably a multitude of parallel and/or serial micro systems. The disposable chips will be filled with sample just before the analysis and is then

25   placed in a reusable apparatus. This is shown in Figure 24 where the disposable chip 88 containing one or more micro systems is placed in the reusable apparatus 89. Preferably, the apparatus comprises means of detection (such as photo multiplier tubes, photo diodes, avalanche photo diodes, potentiostats), means of signal processing (such as amplifiers, filters, integrated circuits) and means of delivering

30   the analysis results to the user (LCD or dot matrix displays, printers or interfaces for computers and/or network).

Definitions

Laminar flow: type of fluid (gas or liquid) flow in which the fluid travels smoothly or in regular paths. The velocity, pressure, and other flow properties at each point in
5   the fluid remain constant.

Turbulence: type of fluid (gas or liquid) movement in which the fluid undergoes ir-regular fluctuations, or mixing (such as a Bérnard cell). The speed of the fluid at a point is continuously undergoing changes in both magnitude and direction.
10

Convection: movement of fluid. Convection can in relation to the present invention describe the situations: (see Fig. 20) 74 laminar flow, 75 buoyancy, inter compart-ment and 76 buoyancy, intra compartment.

15   No convection: fluid does not move. The velocity of points in the fluid is zero, or substantially zero, with respect to the compartment.
The present invention moves particles in a non-moving fluid with a field. The field is the most important contributor to the movement of the particles. Thus, the fluid should not move faster than the particles in the direction of the moving particles.
20

Closed system: system, which does not exchange matter with the surroundings.

non-flow system: System without flow of fluid to and/or from the system.

25   Interconnection is a state, which exists at phase boundaries (interface), which may be established by means of connecting fluids or by means of eliminating a barrier between liquids.

Chip: part which comprises one or more compartments. Chip may be removable
30   from field and/or detection means. The chip may be disposable.

MRM (magnetically responsive material): material, which interacts with a magnetic field, with either repulsive or attractive force. Examples are ferromagnetic, antifer-

romagnetic, paramagnetic, superparamagnetic and superconducting materials. The magnetic interaction of the MRM should be substantially different from the magnetic interaction of the liquid with the field.

5   Non-magnetic: diamagnetic (or has only weak ferromagnetic, antiferromagnetic, paramagnetic or superparamagnetic properties).

LNA: Locked Nucleic Acids

10   PNA: Peptide Nucleic Acid

CLAIMS

1. Micro system comprising a system of operably linked, interconnected compartments wherein at least one reagent immobilised on at least one particle is capable of contacting an analyte comprised in a liquid carrier, said micro system comprising

i) at least one particle with surface properties suitable for immobilising at least one reagent thereon,

ii) at least one reagent suitable for being immobilised on the surface of the at least one particle,

iii) a first compartment preferably for storage of the at least one particle,

iv) a second compartment in which the liquid sample may interact with the reagent immobilised on at least one particle, each of said first and second compartments having at least one opening for passing liquids between the compartment and the exterior,

v) means for subjecting at least part of the system to a field so as to move at least one particle between said first and said second compartment, and

vi) a passage defined between said first compartment and said second compartment so as to allow at least one particle to be moved from one of said compartments to the other through said passage.

2. System according to claim 1, wherein said second compartment further comprises a second opening for passing liquids between said compartment and the exterior.

3. System according to claim 1 or 2, wherein the system comprises at least one field generating means adapted to apply a field to at least a part of the system, and at least one particle being at least partly made from a material susceptible to the generated field.

5

4. System according to claim 3, wherein the generated field is a magnetic field.

5. System according to claim 4, wherein the field generating means comprise at least one electromagnet.

10

6. System according to claim 3, wherein the field generating means comprises two electrodes in electrical contact with the liquid in the system so that the field generating means are activated by applying an electrical potential difference between the two electrodes and at least one particle is moved by electrophoresis.

15

7. System according to claim 3, wherein the field generating means comprises two electrodes which are not in electrical contact with the liquid in the system so that the field generating means are activated by applying an electrical potential difference between the two electrodes and at least one particle is moved by dielectrophoresis.

20

8. System according to claim 3, wherein the field is generated by centrifugation of the system.

9. System according to claim 1 or 2, wherein the field is a gravitational field.

25

10. System according to any of claims 1-9 and further comprising detection means for detecting properties of at least one reagent immobilised on the surface of at least one particle.

30   11. System according to claim 10 and further comprising a third compartment for performing the detection of the properties of at least one reagent immobilised on the surface of at least one particle with the detection means, said third compartment comprising

i) an opening for passing liquids between the compartment and the exterior,

ii) an area that is transparent to allow of optical access from the exterior to the
5   interior of the compartment, and

iii) a passage defined between the second compartment and the third compartment
so as to allow particles to be moved between the second compartment and the third
compartment,

10

iv) the means for subjecting at least a part of the system to a field being adapted for
moving at least one particle between the second compartment and the third
compartment by said field.

15   12. System according to any of claims 1-11 and comprising

i) at least one auxiliary compartment, said auxiliary compartment comprises an
opening for passing liquids between the compartment and the exterior, and

20   ii) a passage defined between the auxiliary compartment and one of the other
compartments so as to allow particles to be moved between said compartment and
the auxiliary compartment,

iii) the means for subjecting at least part of the system to a field being adapted for
25   moving at least one particle between the auxiliary compartment and said
compartment by said field.

13. System according to any of claims 3-12, wherein the generating means of the
system are adapted for moving at least one particle back and forth between
30   compartments between which a passage is defined.

14. System according to any of claims 1-13, wherein one of the compartments is adapted for letting electromagnetic radiation of certain wavelengths reach the liquid contained in said compartment.

5   15. System according to the preceding claim, where the electromagnetic radiation is light.

16. System according to any of claims 1-15, wherein at least one particle is of a mean diameter of 1-200 micro meter.

10

17. System according to any of claims 1-16 wherein the cross-sectional dimensions of said compartments are within 100-1000 micro meter.

18. System according to any of claims 1-17, wherein the system is manufactured

15   from materials that are non-magnetic.

19. System according to any of claims 1-18, wherein the system is manufactured from materials that are non-autofluorescent, such as Topas.

20   20. System according to any of claims 1-19, wherein the interconnections between operably linked compartments are closed until they by activation are opened prior to the performance of a test so as to prevent the contents of the compartments to mix before a test.

25   21. System according to claim 20, wherein the interconnections are closed with a material that is solid before activation, and the activation is performed by heating at least a part of the system of compartments until the closing material becomes liquid.

30   22. System according to claim 20, wherein the activation of the interconnections is performed by physically aligning the compartments.

23. System according to any of the preceding claims, wherein said movement of at least one particle generated by said force is such that substantially no convection of said liquid carrier and said further liquid carrier takes place during particle movement and/or analyte contacting by said reagent

5

24. A method of moving a particle comprising at least one reagent immobilised thereon into a liquid sample that is contained in a micro system comprising a system comprising a plurality of operatively linked compartments, the method comprising the steps of

10

i) providing at least one particle with at least one reagent immobilised thereon, and

ii) entering said particle into a first compartment, and

15 iii) entering a liquid carrier into said first compartment, and

iv) entering a further liquid carrier into a second compartment, and

v) subjecting said micro system to a field exerting a force on at least one particle

20 susceptible to said field, and

vi) moving by means of said force at least one particle from said liquid carrier comprised in said first compartment into said further liquid carrier comprised in said second compartment, wherein said movement of at least one particle generated by

25 said force is such that substantially no convection of said liquid carrier and said further liquid carrier takes place during particle movement and/or analyte contacting by said reagent, and optionally

vii) contacting an analyte comprised in said further liquid carrier with said reagent

30 on at least one particle.

25. Method according to claim 24, wherein said further liquid carrier is a sample for analysis putatively comprising an analyte capable of being contacted by said reagent.

5   26. Method according to claim 24, said method comprising a step of contacting an analyte comprised in said further liquid carrier with said reagent on at least one particle.

27. Method according to claim 24, said method comprising at least one further step

10 of washing and/or purifying and/or detecting said analyte in said first compartment comprising said liquid carrier and optionally an amount of said further liquid carrier that does not interfere with the efficacy of said step of washing and/or purifying and/or detecting said analyte, said method comprising the even further step of moving said reagent contacting said analyte comprised in said sample for analysis

15 comprised in said further liquid carrier, from said further liquid carrier comprised in said second compartment into said first compartment comprising said liquid carrier.

28. A method of moving a particle comprising at least one reagent immobilised thereon into a liquid sample that is contained in a micro system comprising a

20 system comprising a plurality of operatively linked compartments, the method comprising the steps of

i) providing at least one particle with at least one reagent immobilised thereon, and

25 ii) entering said particle into a first compartment, and

iii) entering a liquid carrier into said first compartment, and optionally

iv) contacting an analyte comprised in said liquid carrier with said reagent on at

30 least one particle, and

v) entering a further liquid carrier into a second compartment, and

vi) subjecting said micro system to a field exerting a force on at least one particle susceptible to said field, and

vii) moving by means of said force at least one particle comprising a reagent
5   contacting said liquid carrier comprised in said first compartment into said further liquid carrier comprised in said second compartment, wherein said movement of at least one particle generated by said force is such that substantially no convection of said liquid carrier and said further liquid carrier takes place during particle movement and/or analyte contacting by said reagent.

10

29. Method according to claim 28, wherein said liquid carrier is a sample for analysis putatively comprising an analyte capable of being contacted by said reagent.

15   30. Method according to claim 28, said method comprising a step of contacting an analyte comprised in said liquid carrier with said reagent on at least one particle

31. Method according to claim 28, wherein said further liquid carrier is entered into said second compartment prior to or simultaneously with said analyte in said liquid
20   carrier being contacted with said reagent on at least one particle.

32. Method according to any of the preceding claims, wherein said particle and said liquid carrier are entered into said first compartment either at least essentially simultaneously or sequentially in any order.

25

33. Method according to any of the preceding claims, wherein said particle is preferably disposable and more preferably reconstitutable from a long term storage stable condition such as a frozen, cryoprotected, or freeze dried condition, prior to being entered into said first compartment.

30

34. Method according to any of the preceding claims, wherein said liquid carrier and/or said further liquid carrier is selected from the group consisting of water, saline, any physiologically acceptable aqueous solvent, any pharmaceutically acceptable aqueous solvent, any organic solvent, including any mixture thereof.

5

35. Method according to any of the preceding claims, wherein said reagent is selected from the group consisting of a nucleic acid such as a DNA, RNA or PNA molecule, including any derivative or part thereof, a polypeptide, including any derivative or part thereof including peptides and epitopes, a receptor moiety such

10   as a receptor capable of binding a cell differentiation factor such as a cytokine or a lymphokine, an antibody including a chimeric antibody, a heterodimeric antibody, and a monoclonal antibody, including any binding fragments thereof.

36. Method according to any of the preceding claims, said method comprising at

15   least one further step of washing and/or purifying and/or detecting said analyte contacted by said reagent, with the proviso that said at least one further step does not take place in said first compartment.

37. Method according to any of the previous claims, wherein the step of subjecting

20   the system to a field comprises the step of positioning field generating means for generation of a field that is subjected to at least a part of the system at the system.

38. Method according to any of the preceding claims, wherein the step of subjecting the system to a field comprises the step of generating a magnetic field.

25

39. Method according to claim 37, wherein the step of positioning field generating means at the system comprises positioning an electromagnet at the system and the step of subjecting the system to a field comprises activating the electromagnet with an electric current.

30

40. Method according to claim 37, wherein the step of positioning field generating means at the system comprises positioning two electrodes in electrical contact with the liquid in the system and the step of subjecting the system to a field comprises

the step of supplying an electric potential between the two electrodes so that at least one particle is moved by electrophoresis.

41. Method according to claim 37, wherein the step of positioning field generating
5   means at the system comprises positioning two electrodes at the system in such a way that they are not in electrical contact with the liquid in the system and the step of subjecting the system to a field comprises the step of supplying an electric potential between the two electrodes so that at least one particle is moved by dielectrophoresis.
10

42. Method according to claim 37, wherein the step of subjecting the system to a field comprises the step of centrifugation of the system.

43. Method according to claim 37, wherein the step of subjecting the system to a
15   field comprises the step of subjecting the system to the field of gravitation.

44. Method according to any of claims 37 to 43, said method further comprising the further step of monitoring properties of at least one particle during the interaction.

20   45. Method according to any of claims 37 to 44, further comprising the step of monitoring properties of at least one particle after the interaction.

46. Method according to any of the preceding claims, wherein the system further comprises a third compartment that is interconnected with the second
25   compartment, the method further comprising the step of moving at least one particle by means of the field into the third compartment so as to perform the monitoring of the properties of at least one particle when at least one particle is situated in the third compartment.

30   47. Method according to any of the previous claims, wherein the system further comprises a secondary interaction-compartment that is interconnected with the second compartment, that prior to the step of monitoring further comprises the steps of

i) moving at least one particle by means of the field into the secondary interaction-compartment of the system, and

5   ii) allowing at least one particle to interact with a liquid contained in the secondary interaction-compartment so as to make the result of the interaction between the reagents and the content of the liquid sample detectable by the detection means.

48. Method according to any of the preceding claims, wherein the system further
10   comprises a washing-compartment that is interconnected with any of the other compartments and comprising the steps of

i) moving at least one particle into the washing-compartment of the system by means of the field, and

15

ii) allowing at least one particle to interact with a liquid contained in the washing-compartment so as to remove unwanted material from at least one particle.

49. Method according to any of the preceding claims, wherein one of the
20   compartments is adapted for letting electromagnetic radiation of certain wavelengths reach the liquid contained in said compartment, the method further comprises the step of subjecting at least one particle to electromagnetic radiation of a wavelength suitable for causing a process of photoactivation.

25   50. Method according to any of the preceding claims, wherein at least one particle is of a mean diameter of 1-200 micro meter.

51. Method according to any of the preceding claims, wherein the cross-sectional dimensions of said compartments are within 100-1000 micro meter.

30

52. Method according to any of the preceding claims, wherein said analyte is a biological organism, or a part thereof, selected from the group consisting of a cell, an infectious agent including a virus, and a parasite, including any part or combination thereof.

5

53. Method according to claim 52, wherein said organism is a mammalian organism such as a human or animal organism, such as a human or animal cell, including any derivative thereof, or a virus or parasite, including any parasitic fungi, capable of being harboured in or replicated in a human or animal cell or a derivative thereof.

10

54. Method according to claim 53, wherein said cell, virus or parasite is pathogenic or potentially lethal.

55. Method according to claim 52, wherein said organism is a microbial organism

15   such as a eukaryotic or prokaryotic microbial organism.

56. Method according to claim 55, wherein said microbial organism is a pathogenic or a potentially lethal microbial organism.

20   57. Method according to any of the preceding claims, wherein said analyte is an antigen or an antibody indicative of a predetermined cell type.

58. Method according to any of the preceding claims, said method comprising the further step of performing in at least one of said compartments a method of

25   amplifying a biological compound by a plurality of thermo cyclic reactions at predetermined temperatures, such as reactions suitable for annealing nucleic acids, extension reactions suitable for synthesising a nucleic acid, and denaturing reactions suitable for separating synthesised double stranded nucleic acids.

30   59. Method according to any of claims 24 to 58, wherein said micro system is the system according to any of claims 1 to 23.

60. Method of diagnosing a condition in an individual by detecting an analyte in a sample,

said diagnostic method comprising providing a sample from said individual and a

5 method of detecting in said sample an analyte, the presence of which is an indication of said individual having contracted said condition,

said method of detection further comprising the steps of

10 i) moving a particle comprising at least one reagent immobilised thereon into a liquid sample that is contained in a micro system comprising a system comprising a plurality of operatively linked compartments according to the method according to any of claims 24 to 59, and

15 ii) contacting said reagent with said analyte comprised in a sample in the form of said liquid carrier or said further liquid carrier for the purpose of

iii) detecting diagnostically said analyte contacted by said reagent, and

20 iv) Diagnosing said condition.

61. Method of analysing the content of a liquid contained in a container, said method comprising the steps of

25 (a)   mixing particles with the liquid so as to obtain a substantially even distribution of the particles in at least a part of the liquid, the particles being at least partly made from a material susceptible to a field, such as a magnetic field or an electric field, and having at least one reagent immobilised on a plurality of the particles,

30

(b)   allowing the reagent immobilised on the particles to interact with the content of the liquid,

(c) applying a field to which the particles are susceptible to at least a part of the container so as to move at least one of the particles through an opening of the container to extract at least one particle from the container,

5 (d) moving at least one particle through a liquid-filled passage to detection means for detecting properties of the reagents on said at least one particle, and

(e) detecting properties of the reagent on said at least one extracted particle in order to determine whether these properties have changed due to the
10 interaction, so as to perform an analysis of the liquid.

62. Method according to claim 61, wherein the steps (c) to (e) are repeated at least once after elapse of a predetermined time period so as to provide a monitoring of a possible ongoing process involving the liquid.

15

63. A system for distinguishing between particles with different magnetic properties, the system comprising

first detection means, such as a Hall sensor, for detection of the magnetic
20 properties of particles and adapted to provide an output according to the magnetic properties of a particle comprised within a first measuring volume of the first detection means,

a population of particles made at least partly from a material with magnetic
25 properties that are detectable by the first detection means, the population of particles comprising at least two subpopulations of particles, each subpopulation of particles having different magnetic properties so that said output from the first detection means may provide a significant indication of which subpopulation a detected particle is a member of,

30

a liquid in which the particles are contained when the magnetic properties of the particles are being detected by the first detection means, and

a member with a flow channel defined therein for leading the liquid with the particles contained therein through the first measuring volume, the flow of the liquid being controlled in such a way that one particle at a time passes the first measuring volume.

5

64. A system according to claim 63, wherein the particles have surface properties suitable for immobilising at least one reagent thereon and reagents are immobilised on a substantial number of the particles within each subpopulation so that each subpopulation has a specific reagent assign to it and at least two of the

10 subpopulations have different specific reagents assigned to them, so as to enable performance of an analysis with at least two different specific reagents of the content of a liquid sample.

65. A system according to claim 63 and comprising second detection means for

15 detecting properties of reagents immobilised on particles comprised within a second measuring volume of the second detection means, for determining whether said properties have changed during an interaction between the content of the liquid sample and the particles, so as to perform an analysis of the content of the liquid sample.

20

66. A system according to claim 65, wherein the second measuring volume is positioned and the flow of the liquid with the particles contained therein is controlled in such a way that particles passing the first measuring volume one at a time also passes the second measuring volume one at a time.

25

67. A system according to any of claims 63 to 66, wherein the flow channel defined in the member comprises means for entering at least one buffer-liquid into the flow channel parallel to the flow of the liquid with the particles contained therein, for controlling the flow in such a way that particles pass the first and optionally the

30 second measuring volume one particle at a time.

1/18
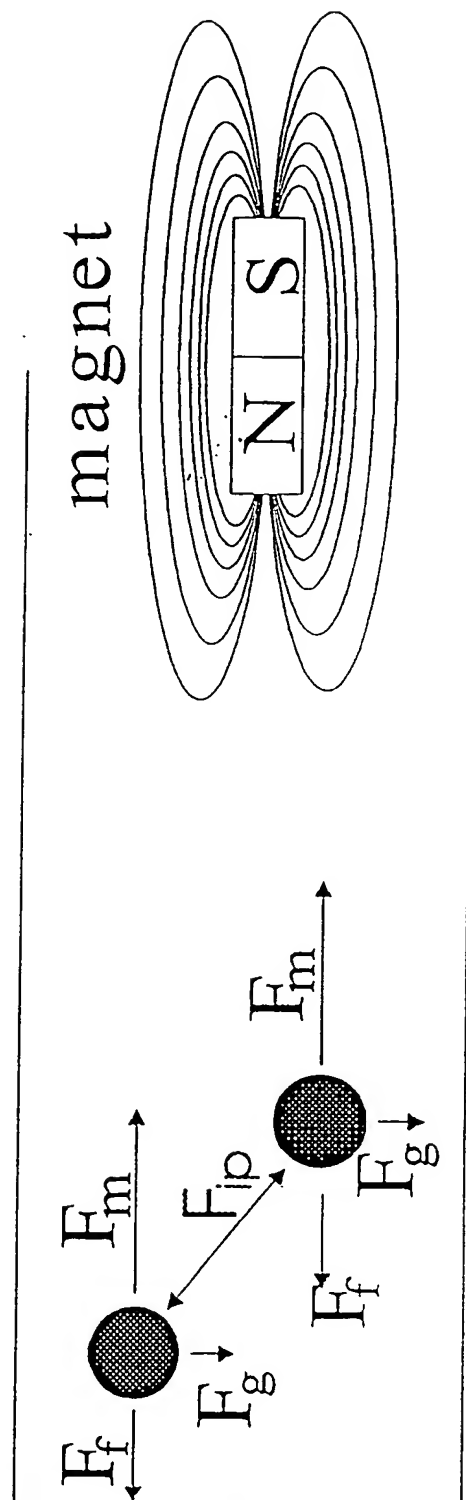
Fig. 1
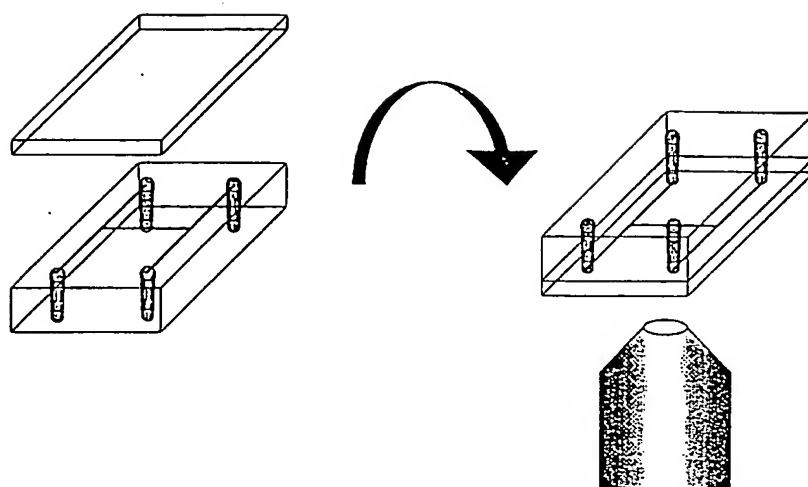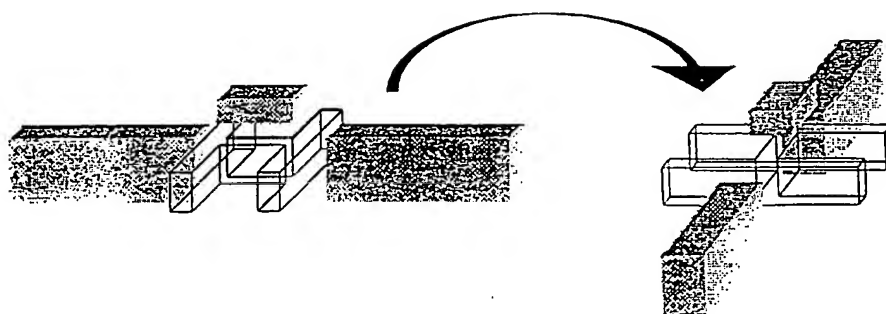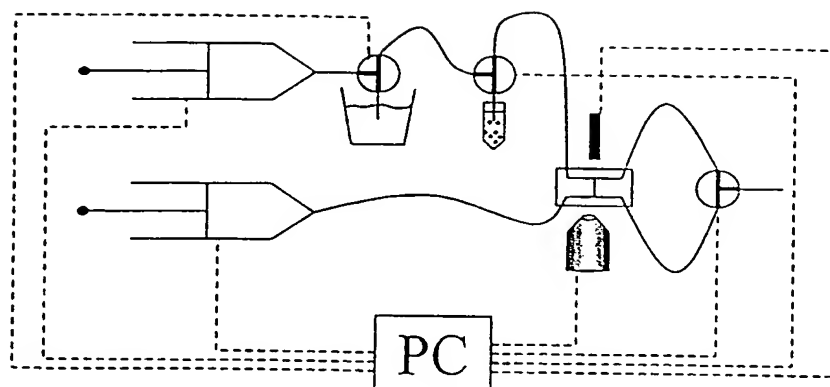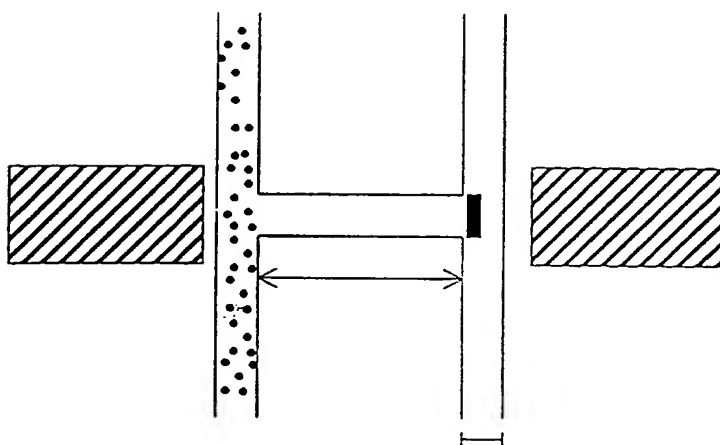
Fig. 2

Fig. 3

Fig. 4

Fig. 5

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Fig. 6A

Fig. 6B

on

Fig. 6C

on

Fig. 6D

on

di-aggregates

mono disperse

time of flight

Fig. 7

peak height

peak width

Time of flight
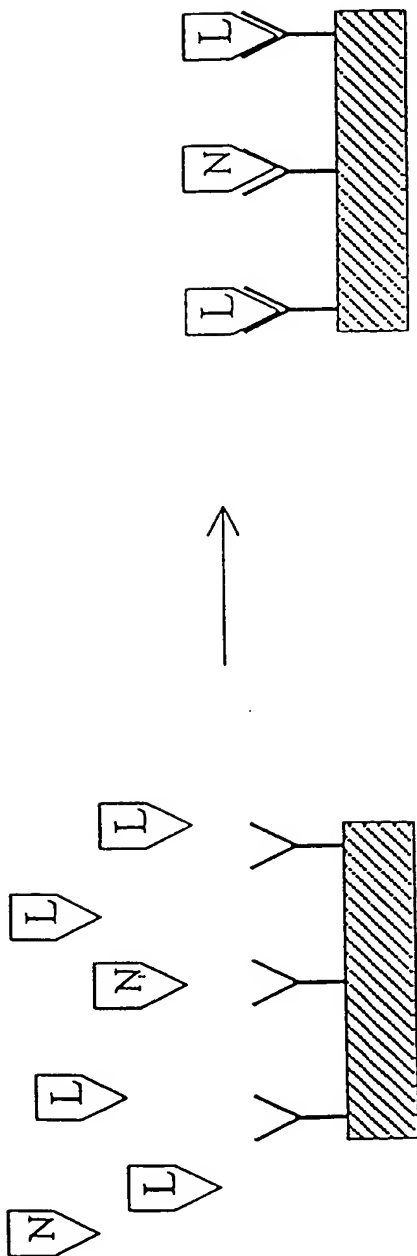
credible peaks
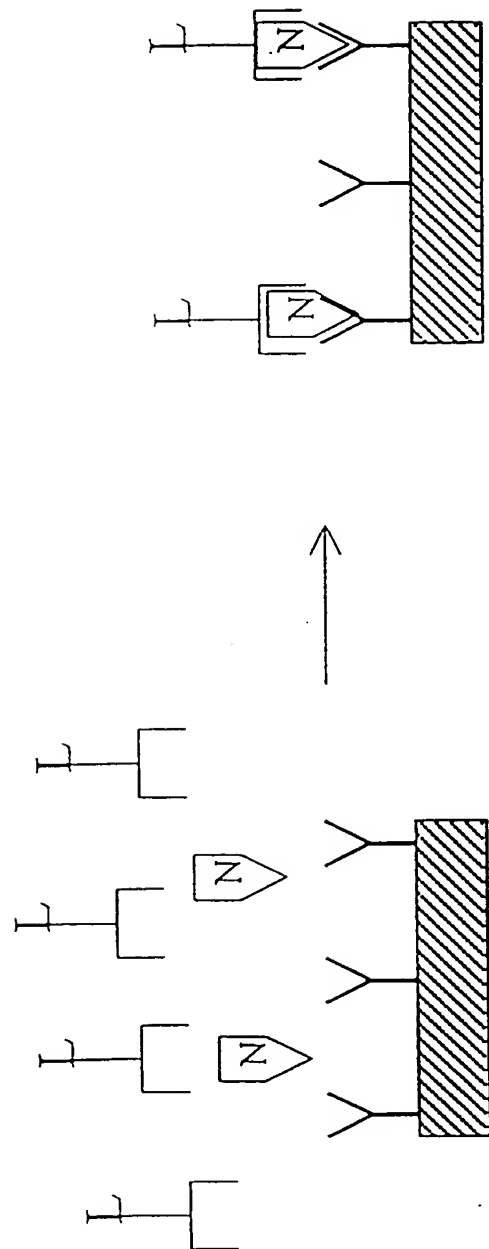
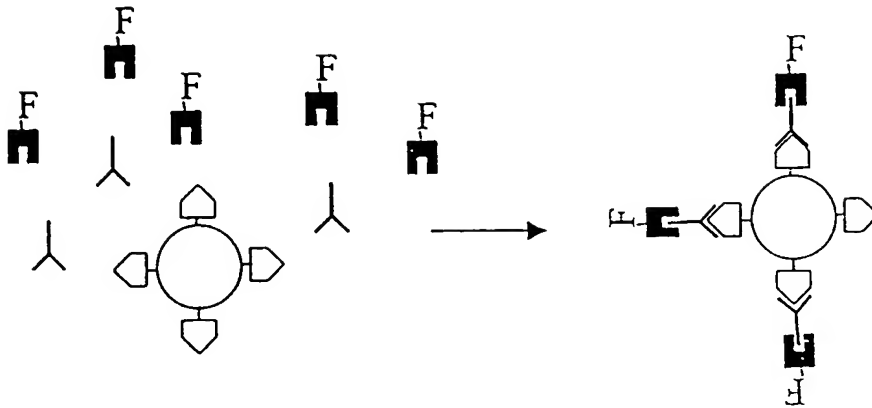mono disperse

forbidden peaks

Fig. 8

di-aggregates

peak width
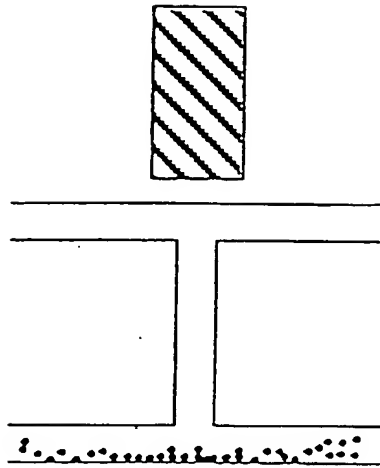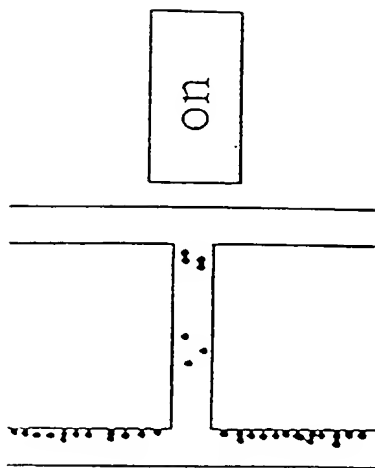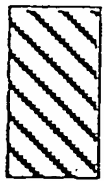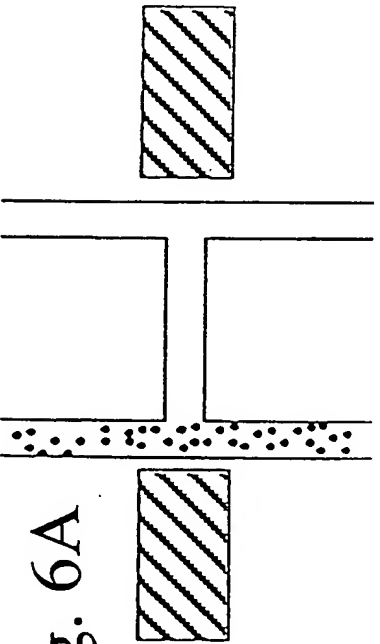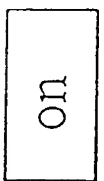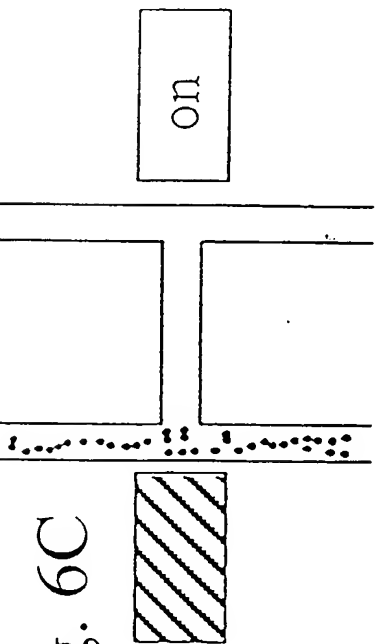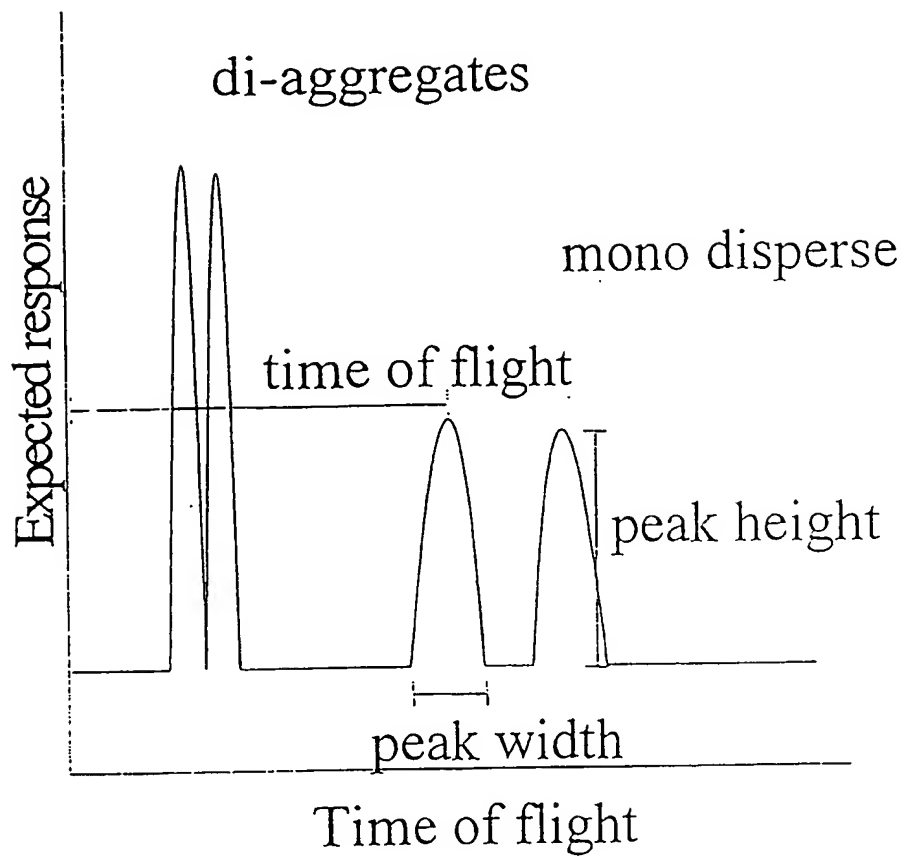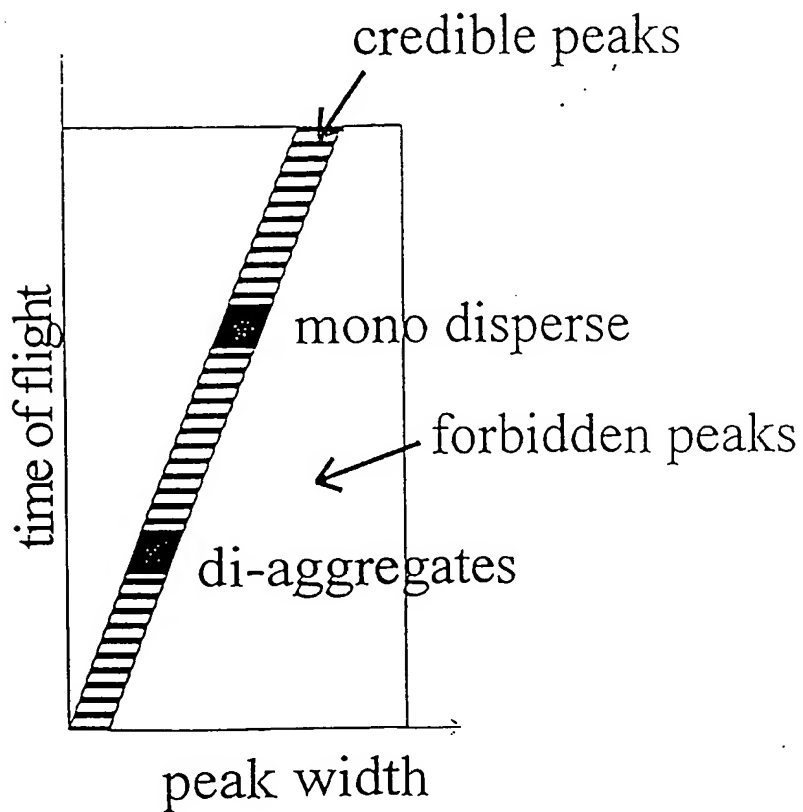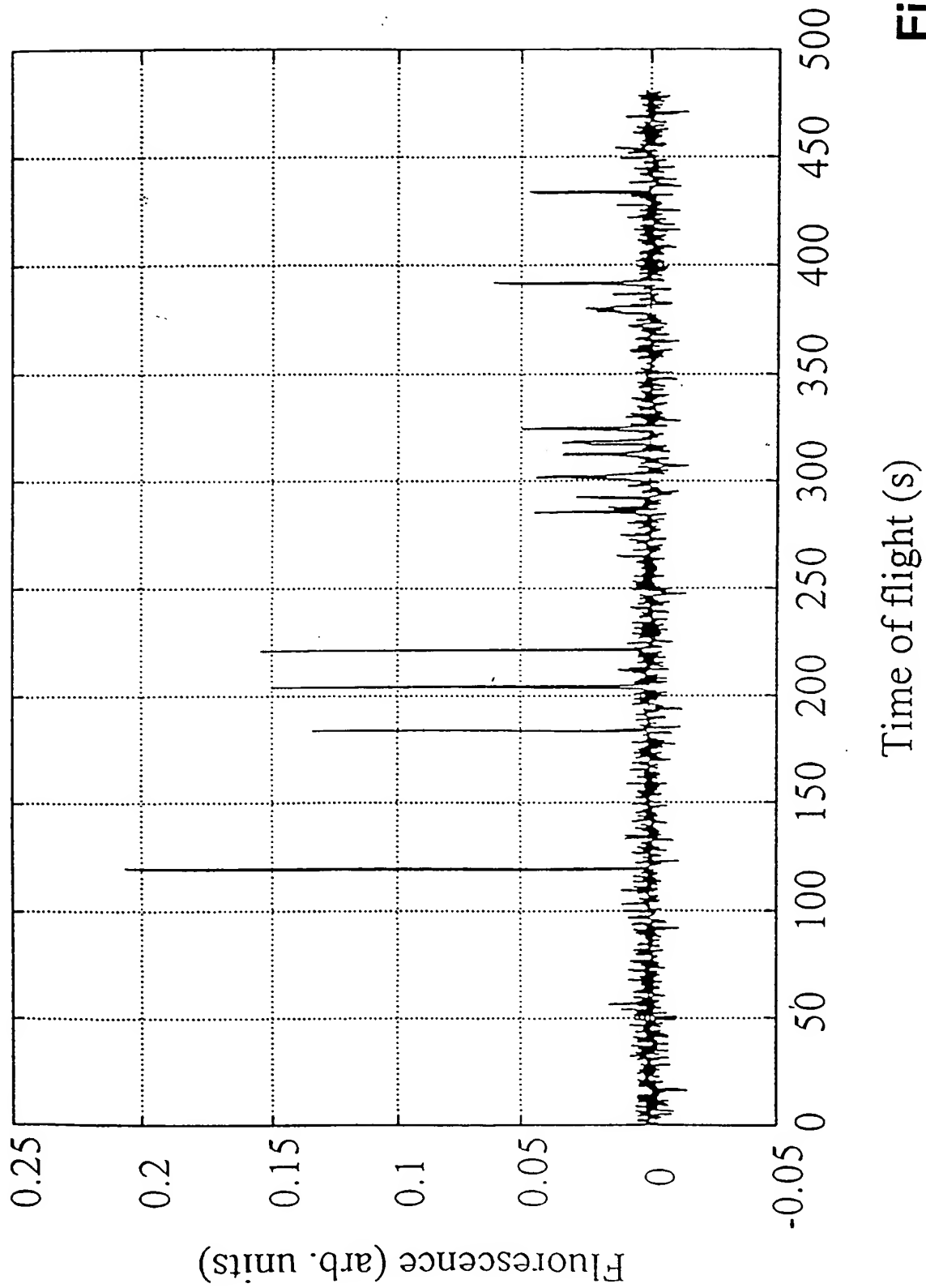
Fig. 9

Fig. 10a

Fig. 10b

Fig. 11

Fig. 12a

Fig. 12b

Fig. 13

Fig. 14

10/18

Fig. 15

11/18

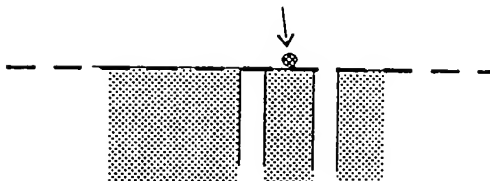Fig. 16A Fig. 16B Fig. 16C

Fig. 16D Fig. 16E Fig. 16F

Light
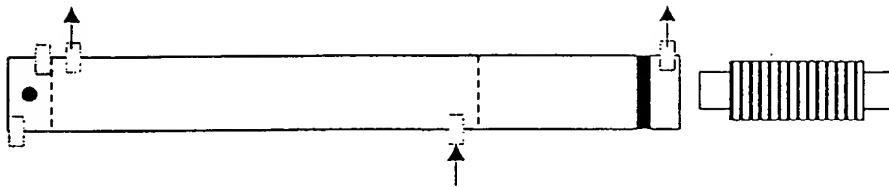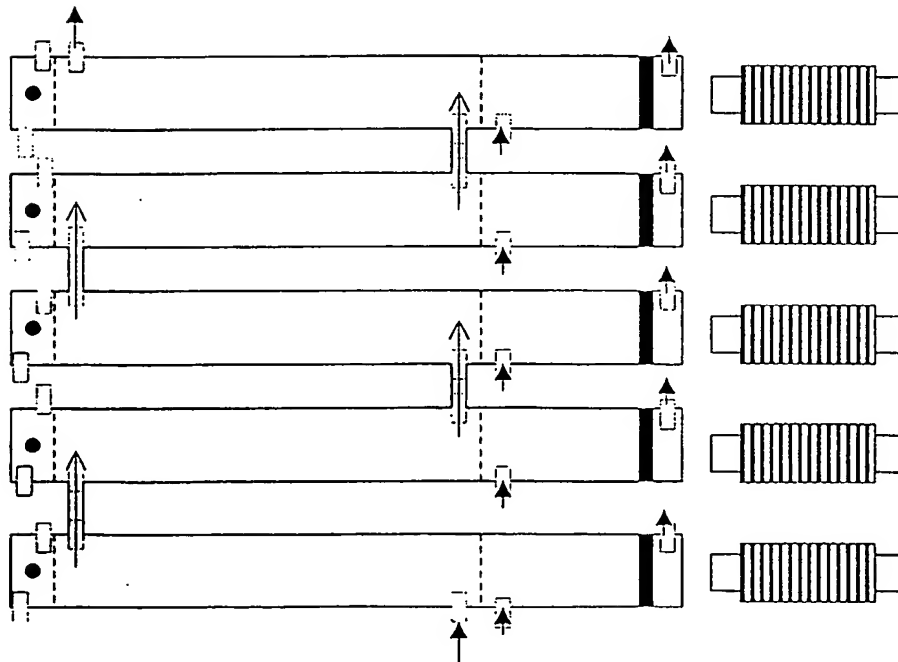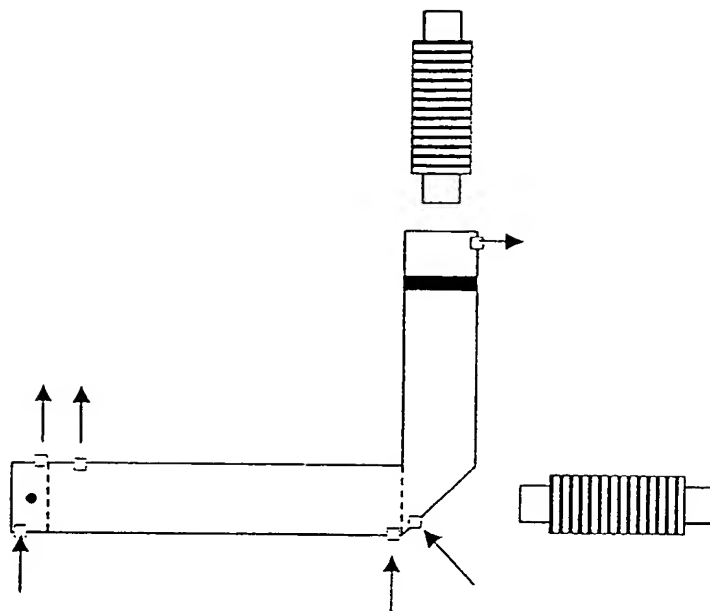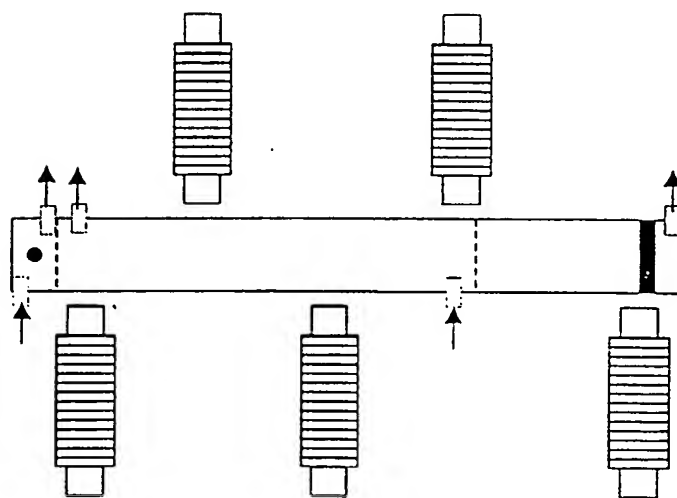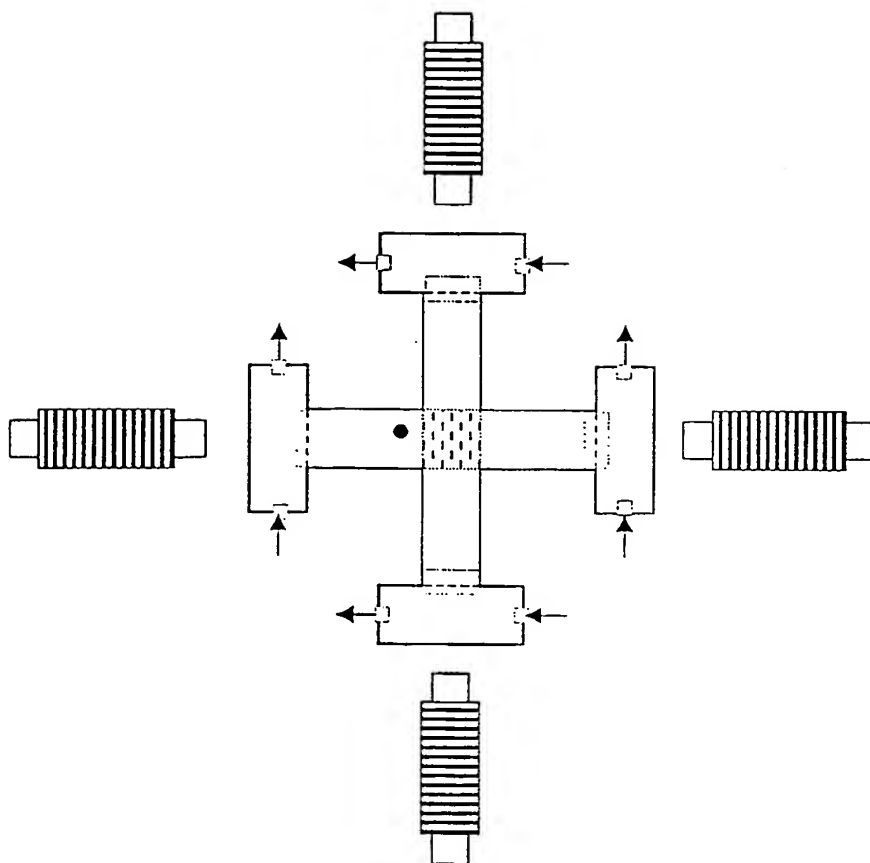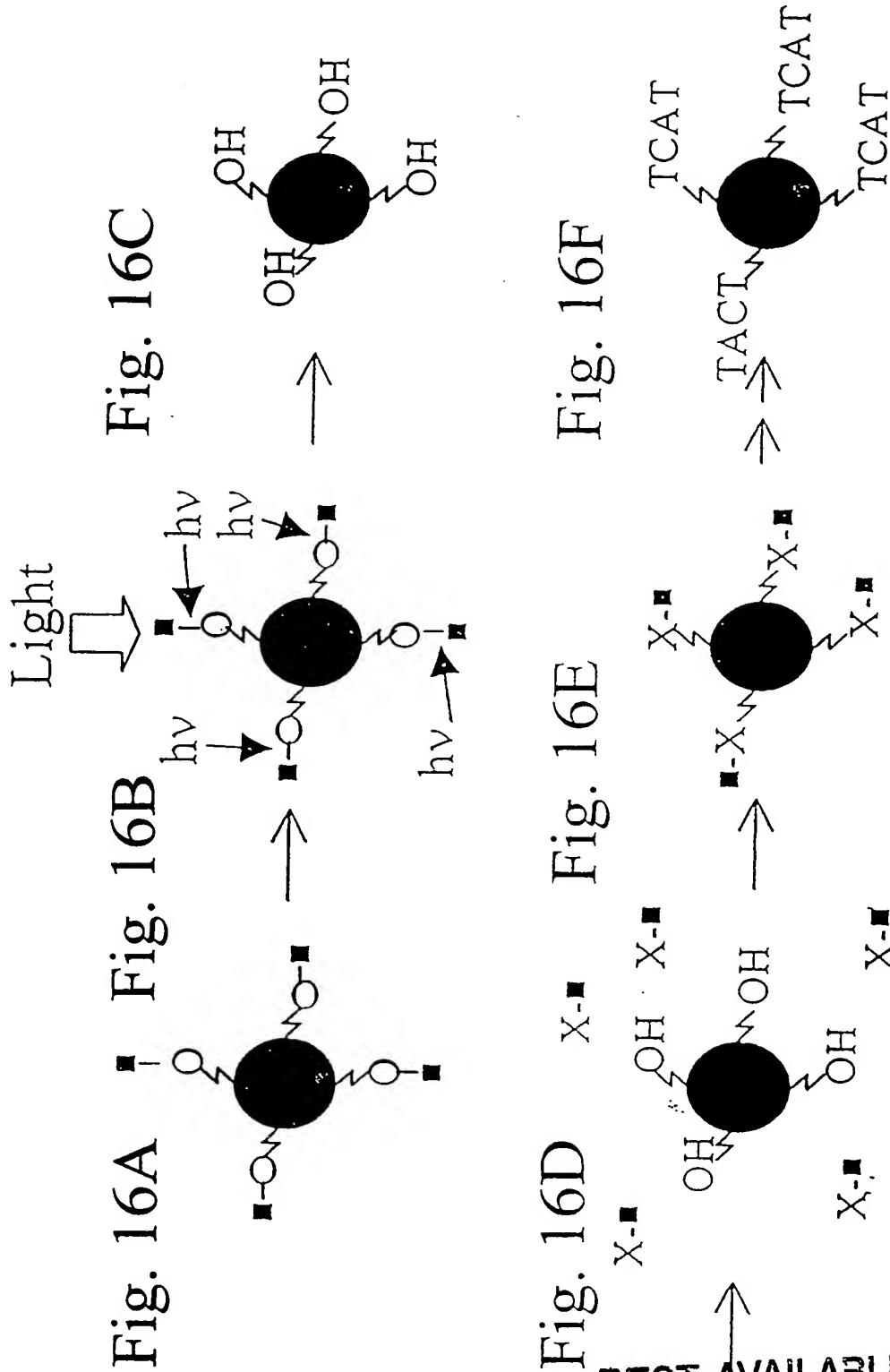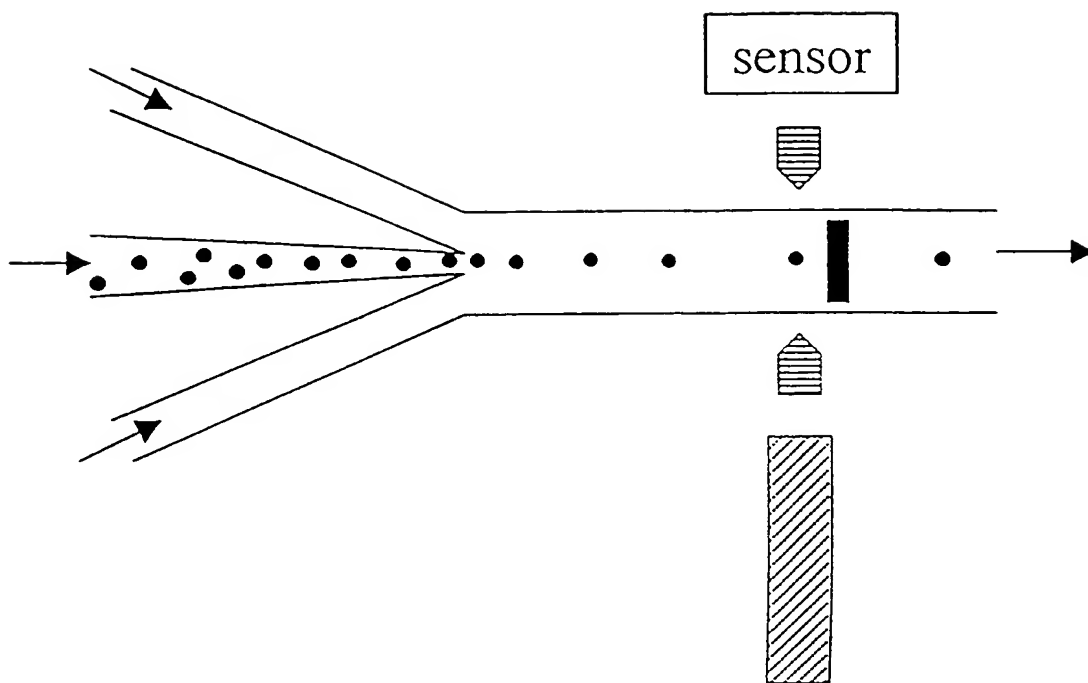
Fig. 17
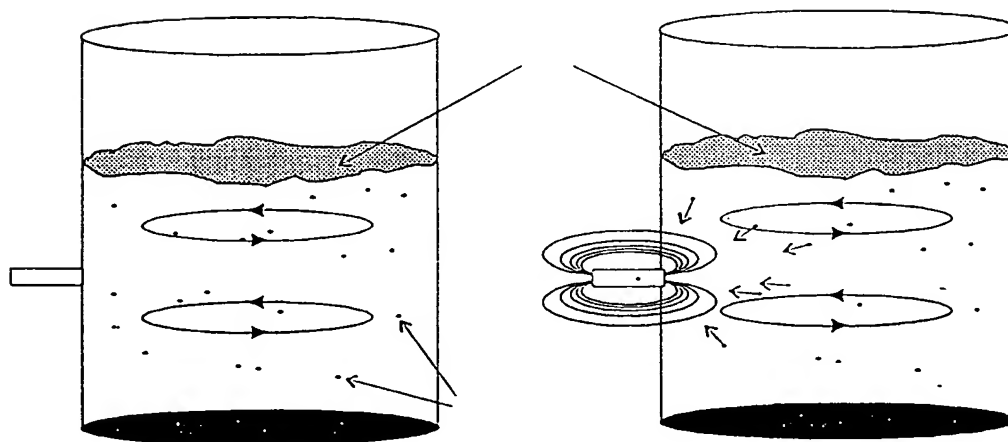
Fig. 18

Fig. 19c

Fig. 19b

Fig. 19a
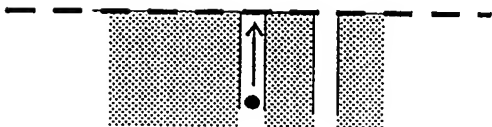
Fig. 20
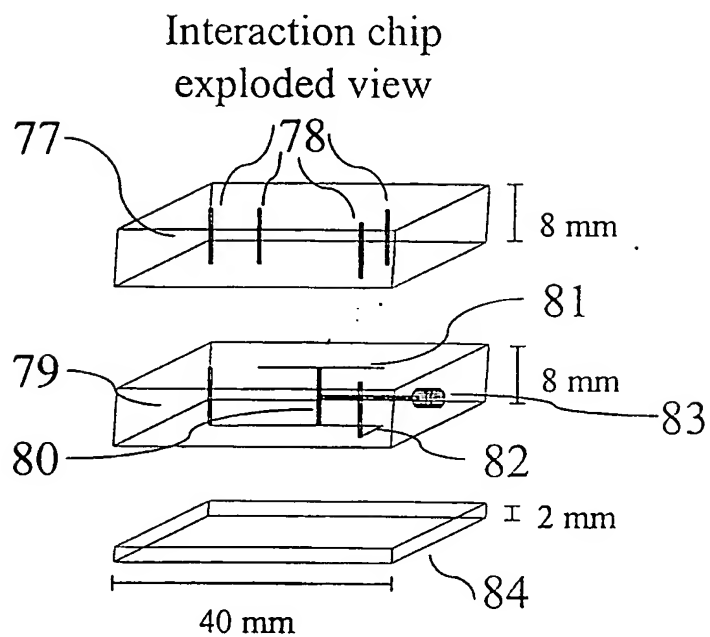
Figure 21.A
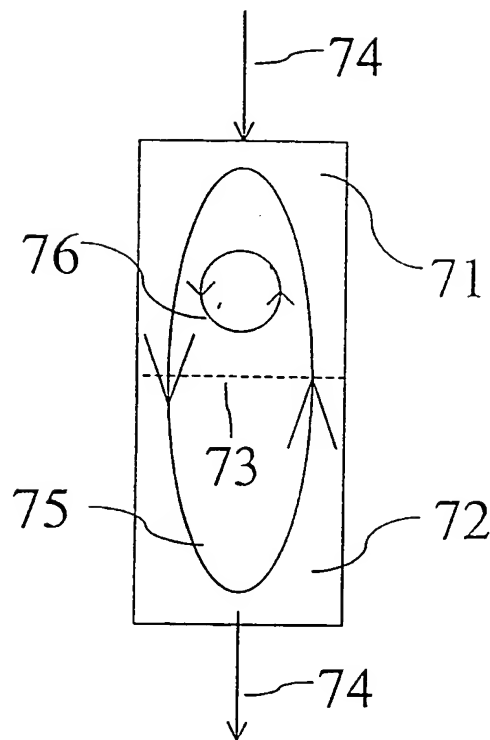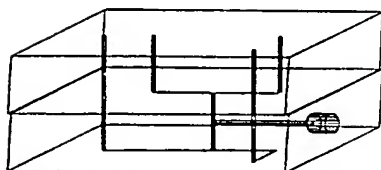
Interaction chip
exploded view

Figure 21.B
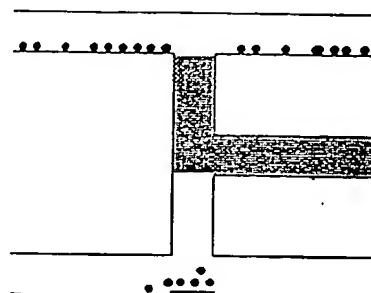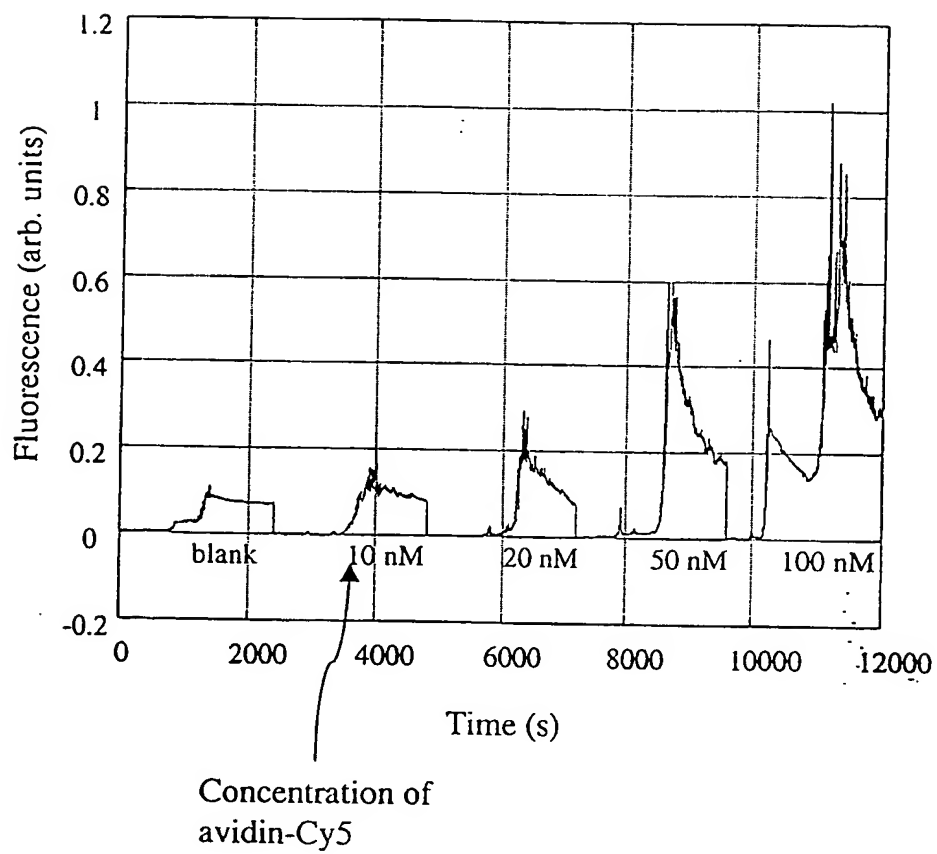
Interaction chip
as used
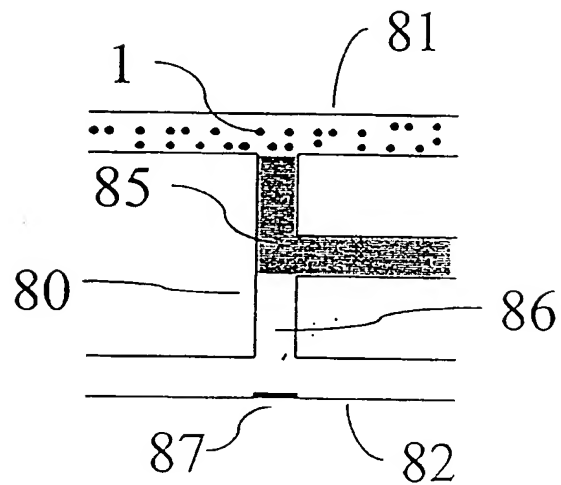
Figure 22.A

Figure 22.B

Figure 22.C

Figure 23

Concentration of
avidin-Cy5
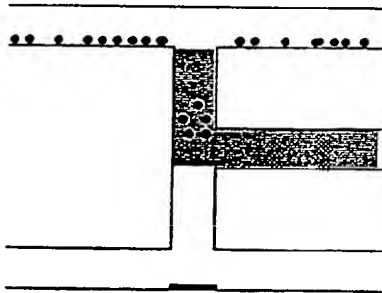
Figure 24

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6   G01N35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6   G01N B01L B03C C12Q B01J C12M

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 10267 A (BLANKENSTEIN GERT ;TECHNICAL UNIVERSITY OF DENMAR (DK)) 12 March 1998	1-5,7,9, 10,12, 14,15, 17-19, 61-67
A	see page 5, line 8 - page 5, line 34	24,35
A	see page 6, line 8 - page 6, line 12	51-53
A	see page 7, line 25 - page 9, line 20	39-41,45
	see page 10, line 25 - page 10, line 29	
	see page 11, line 23 - page 11, line 28	
A	see page 12, line 4 - page 12, line 7	54
A	see page 14, line 16 - page 14, line 27	60
A	see page 18, line 29 - page 20, line 22	31,32, 37,38, 43,44
A	see page 21, line 8 - page 21, line 16	40
A	see page 21, line 36 - page 23, line 28	46,47
A	see page 23, line 34 - page 23, line 37	25-27,36
A	see page 25, line 36 - page 26, line 27	28-30,
	-/--	

[X] Further documents are listed in the continuation of box C.   [X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
17 June 1999	25/06/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Koch, A

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	see page 28, line 5 - page 28, line 17 see page 33, line 23 - page 34, line 19	45,58,60 48,49,59
A	see page 34, line 29 - page 35, line 9 see figures 2,3,5-7,9 see figures 11,12,14,15 ---	55-57
X	WO 93 24231 A (BIOSITE DIAGNOSTICS INC) 9 December 1993 & US 5 885 527 A (BUECHLER K. F.) 23 March 1999 see column 1, line 23 - column 1, line 28 see column 4, line 43 - column 4, line 57 see column 5, line 1 - column 7, line 14 see column 7, line 53 - column 8, line 16 see column 8, line 59 - column 8, line 65 see column 10, line 40 - column 11, line 2 see column 12, line 32 - column 13, line 6 see column 21, line 63 - column 22, line 36 see figures 1-4 ---	1-3,9, 10,12, 16,17,20
X	WO 93 22053 A (UNIV PENNSYLVANIA) 11 November 1993 see page 5, line 8 - page 6, line 2 see page 7, line 21 - page 9, line 25 see page 18, paragraph 1 see page 20, line 4 - page 20, line 32 see page 22, paragraph 2 - page 24, paragraph 1 see page 25, paragraph 2 - page 26, paragraph 1 see figures 1-18 ---	61-65
P,A	WO 98 46438 A (BOUSSE LUC ;CHOW CALVIN Y H (US); KNAPP MICHAEL R (US); PARCE J WA) 22 October 1998 & US 5 885 470 A (BOUSSE LUC ET AL) 23 March 1999 see column 1, line 28 - column 2, line 43 see column 3, line 5 - column 3, line 25 see column 3, line 61 - column 4, line 34 see column 6, line 52 - column 7, line 34 see column 10, line 26 - column 12, line 44 see column 13, line 44 - column 14, line 29 see figures 1,2 -----	1-3,5,6, 12,17, 19,20, 24-26, 34,35, 37,40, 45,51, 52,59,60

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9810267	A	12-03-1998	AU	4113297 A	26-03-1998
			NO	991051 A	27-04-1999
WO 9324231	A	09-12-1993	US	5458852 A	17-10-1995
			AU	4596593 A	30-12-1993
			EP	0596104 A	11-05-1994
			JP	6509424 T	20-10-1994
			US	5885527 A	23-03-1999
WO 9322053	A	11-11-1993	US	5304487 A	19-04-1994
			US	5296375 A	22-03-1994
			AT	155711 T	15-08-1997
			AT	167816 T	15-07-1998
			AT	140025 T	15-07-1996
			AT	140880 T	15-08-1996
			AT	174813 T	15-01-1999
			AU	677780 B	08-05-1997
			AU	4222393 A	29-11-1993
			AU	680195 B	24-07-1997
			AU	4222593 A	29-11-1993
			AU	677781 B	08-05-1997
			AU	4222693 A	29-11-1993
			AU	4222793 A	29-11-1993
			AU	677197 B	17-04-1997
			AU	4223593 A	29-11-1993
			CA	2134474 A	11-11-1993
			CA	2134475 A	11-11-1993
			CA	2134476 A	11-11-1993
			CA	2134477 A	11-11-1993
			CA	2134478 A	11-11-1993
			DE	69303483 D	08-08-1996
			DE	69303483 T	06-02-1997
			DE	69303898 D	05-09-1996
			DE	69303898 T	20-02-1997
			DE	69312483 D	04-09-1997
			DE	69312483 T	12-02-1998
			DE	69319427 D	06-08-1998
			DE	69319427 T	10-12-1998
			DE	69322774 D	04-02-1999
			DE	69322774 T	17-06-1999
			EP	0637996 A	15-02-1995
			EP	0637997 A	15-02-1995
			EP	0639223 A	22-02-1995
			EP	0637998 A	15-02-1995
			EP	0637999 A	15-02-1995
			ES	2106341 T	01-11-1997
			ES	2127276 T	16-04-1999
			GR	3025037 T	30-01-1998
			HK	16897 A	13-02-1997
			JP	7506430 T	13-07-1995
			JP	7506431 T	13-07-1995
			JP	7506256 T	13-07-1995
			JP	7506257 T	13-07-1995
			JP	7506258 T	13-07-1995
			WO	9322054 A	11-11-1993
			WO	9322421 A	11-11-1993
			WO	9322055 A	11-11-1993
			WO	9322058 A	11-11-1993

Form PCT/ISA/210 (patent family annex) (July 1992)

Intel onal Application No
PCT/IB 99/00522

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9322053	A		US	5635358 A	03-06-1997
WO 9846438	A	22-10-1998	US	5885470 A	23-03-1999
			AU	7248498 A	11-11-1998